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A SYSTEMS ANALYSIS OF THE ERYTHROPOIETIC RESPONSES TO WEIGHTLESSNESS

VOL. I. MATHEMATICAL MODEL SIMULATIONS OF THE ERYTHROPOIETIC
RESPONSES TO WEIGHTLESSNESS

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Houston, TX

1985

ABSTRACT

A Systems Analysis of the Erythropoietic Responses to Weightlessness

Beginning in 1974 and continuing to the present, a systems analysis research program has focused on the hematological problems of space flight. Specifically, it was desired to understand the mechanisms underlying the most significant hematological finding of clinical importance; that is, the reduction in red cell mass. The cornerstone of this analysis was the development and testing of a mathematical model describing the regulation of erythropoiesis. Simulations performed with this model were used to examine the theoretical behavior of erythropoietic regulation, to provide a means to predict observable findings from space-flight and ground-based studies, and to permit hypotheses to be mathematically tested as a preliminary step in accounting for the "anemia of space flight."

This document contains several major sections, including a review of previous space-flight findings, the role of tissue oxygenation, and other theoretical considerations of erythropoiesis, hypotheses accounting for the effects of space flight, and a review of the computer simulations of experimental studies. Part of the study included a valuable collaboration between systems analysts and biological researchers, which permitted testing, in the biological system, hypotheses suggested by the computer model. Results from a number of experimental studies, each characterized by a reduced red cell mass, or suppressed erythropoietic activity, were examined. These included not only space-flight investigations, but also those of bed rest, red cell infusions, dehydration, and descent-from-altitude. The simulation model was valuable in revealing the pathways which were common to all of these situations and provided a quantitative basis for testing whether the same mechanisms were operative in space flight.

The general conclusion reached during this study was that the loss of red cell mass during space flight was likely a result of suppressed erythropoietic activity rather than elevated destruction of red cells. According to current concepts embodied in the theoretical model, a reduced erythropoietic state can be caused indirectly by hyperoxia of a renal oxygen sensor via the humoral regulator, erythropoietin, acting on the bone marrow, or more directly, by alteration of stem cell kinetics at the marrow controller. Tissue hyperoxia may be caused by factors which increase oxygen supply (i.e., hemoconcentration, enhanced blood flow, decreased oxy-hemoglobin affinity, and increased arterial oxygen loading), or by factors which decrease oxygen demand (decreased basal or physical activity). Proliferation of erythrocytes at the bone marrow level is affected, not only by erythropoietin, but also by dietary factors. One can reasonably postulate that all of these various influences were present to various degrees during space flight and bed rest, and they contributed to the eventual reduction in red cell mass.

The hypothesis most favored is that shifts in plasma volume accompanying hypogravic maneuvers results in an observed mild hemoconcentration which can

eventually lead to increased oxygen supply to the renal sensor and cause suppression of erythropoietin and red cell production. The model suggests that red cell mass will stabilize as hematocrits normalize. This process can, therefore, be explained in terms of normal feedback regulation of the erythropoietic system in the face of sustained decreases in plasma volume. Validation of this theory will require, at the very least, confirmation that erythropoietin levels decrease during space flight. Other factors which may have enhanced oxygen delivery may have included shifts in blood flow, P50, and arterial oxygen partial pressure, but no data exist at present concerning these effects. Also, it is believed that inadequate dietary intake or exercise can also suppress red cell mass for reasons that are not well understood. Alternatively, it is possible that an acute increase in red cell destruction could have occurred during flight (i.e., hemorrhage, hemolysis, sequestration). However, the model suggests that this factor would be contributory to, but not responsible for, the overall zero-g loss of red cell mass.

The concept that red cell mass may be regenerated during space flight, as proposed for the Skylab missions, was examined in detail, and found to be based on misleading assumptions and incomplete interpretation of the data. However, still lacking is a clear understanding of why the three Skylab crews differed in their overall decrements in red cell mass.

For the present, it appears that an answer to the loss of red cell mass will involve an understanding of the basic processes of erythropoiesis, including the role of oxygen transport, as well as the regulation of plasma volume and total blood volume. Subtle changes in energy balance and water balance also need to be carefully controlled in order to isolate the basic mechanisms. An erythropoietin assay with improved resolution is also required to obtain definitive data regarding this important hormonal regulator.

VOL. I. MATHEMATICAL MODEL SIMULATIONS OF THE ERYTHROPOIETIC
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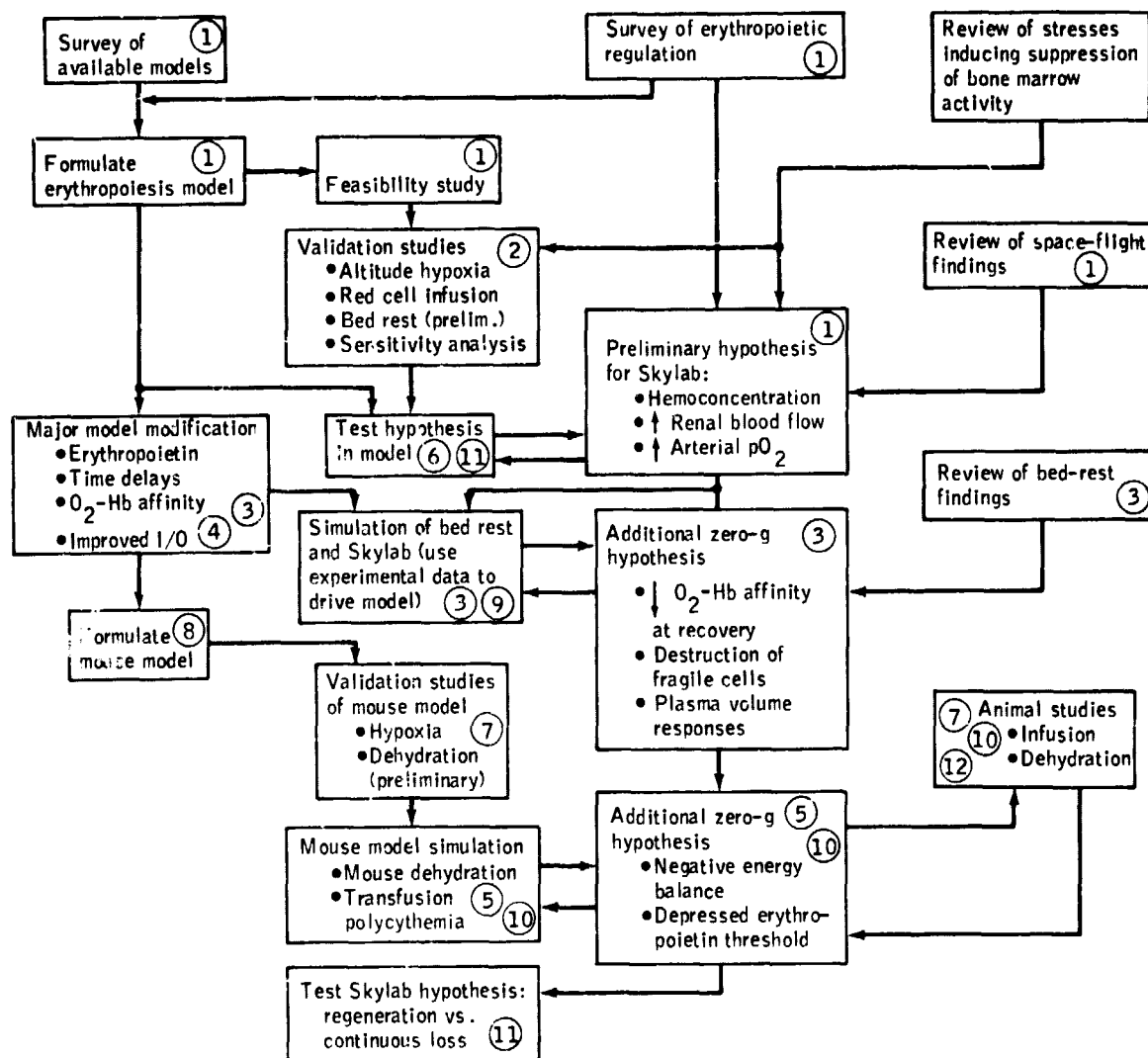
The primary influence of the weightless environment of space flight on the human hematologic system is a reduction in the circulating blood volume during flight. An invariable finding throughout the manned space-flight program has been the occurrence of significant volume shifts in both the red cell and plasma portions of the blood. Previous publications have discussed the key role of decreased total blood volume in the physiological responses to weightlessness (Hoffler, 1977; Leonard et al., 1977; Leonard, 1983). Also, the mechanisms were reviewed by which the plasma volume decrement may have occurred. In this document the emphasis will be on the important factors which may have led to changes in the red cell component of blood. Inasmuch as the red cells provide a dominant role in the oxygen transport functions of the body, the importance of tissue oxygenation will also be considered.

Explanations for the reduction in red cell mass (RCM) have been proposed, but confirmatory evidence is still lacking. The so-called "anemia of space flight" has been variously ascribed to weightlessness, the relative immobility of the crew, lack of atmospheric nitrogen, and a high oxygen partial pressure within the space vehicle (Dunn, 1978; Fisher and Kimzey, 1971; Jensen, 1972; Larkin et al., 1972). There are indications that these factors are involved in altering both destruction and production rates of red cells, not only in space-flight studies, but also in related ground-based experiments using human and animal subjects.

In support of NASA hematology research, the findings and hypotheses of previous space-flight and experimental studies were integrated within the framework of a theoretical model of erythropoiesis regulation. This model, described fully in Volume II of this report (Leonard, 1985) and in Leonard et al. (1981), was developed especially to investigate the agents responsible for the loss of circulating red blood cells observed in crewmen returning from space missions. The studies described in the present document, based on simulations of the mathematical model, provided new insights into and understanding of this phenomenon, which is still under intense investigation.

The general approach for providing systems analysis support of the hematology program is illustrated in Figure 1. The major achievements of the analyses are indicated, as are the reports which describe them. At the outset

MODEL DEVELOPMENT SIMULATION STUDIES HYPOTHESIS DEVELOPMENT EXPERIMENTAL FINDINGS



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- | | |
|------------------------|-------------------------|
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| 2 Leonard 1976 | 8 Leonard 1978 |
| Leonard et al. 1981 | 9 Kimzey et al., 1979 |
| 3 Leonard 1977a | 10 Dunn et al., 1981 |
| 4 Grounds 1978 | 11 Leonard et al., 1977 |
| 5 Leonard et al., 1979 | Leonard 1983 |
| 6 Kimzey et al., 1976b | 12 Dunn and Lange 1979b |

Figure 1: Approach for studying erythropoietic regulation during space flight. The progressive development in each systems analysis area such as model development is in the downward direction of the diagram while the horizontal lines indicate the high degree of interaction between all phases of the study. Hypotheses were formulated based on new insights from the different experimental studies and ultimately the most promising of these were tested against the Skylab data. Numbers in circles refer to published reports.

of the study, a suitable model of erythropoiesis regulation did not exist. In addition, it was uncertain if the systems analysis approach would be of value in this particular investigative area. Therefore, a feasibility study was performed which consisted of a review of the essential physiology describing erythropoiesis regulation, a survey of available models of this system, the formulation and implementation of a preliminary version of a new mathematical model, a review of the space-flight findings, and finally, the development of several simple hypotheses, based on the model's behavior, which could explain these findings. This initial study indicated that the modeling approach might be limited as an investigative tool in the Skylab hematological experiments because of the paucity of inflight data, and because of an incomplete understanding of the mechanisms affecting erythropoiesis. Nevertheless, it was concluded that a more advanced model could assist investigators in several ways that have been previously enumerated in this document (i.e., it could identify important parameters, provide a method by which to test hypotheses rapidly, define areas requiring further study, and predict difficult-to-measure parameters).

The development of the model proceeded in a systematic fashion, as indicated in Figure 1. Verification and validation of the model have been described in Volume II (Leonard, 1985). Simulations were performed for experimental stresses related to space flight such as red blood cell infusions, descent from altitude; and bed rest. Experimental data for these studies were taken from available literature. In addition, collaboration with NASA investigators of bed rest and other zero-g analogs provided specific data that were needed to define model parameters and confirm model predictions. Major improvements were made in the model in order to provide more realistic simulations of these experiments. These included the addition of explicit elements representing erythropoietin and red blood cell production, bone marrow time delays, variations in the oxy-hemoglobin dissociation function curves, and an algorithm for entering experimental data as time-varying driving functions. Other, more generalized simulation analyses, including sensitivity analysis provided information regarding the relative importance of model parameters and some basic quantitative relationships between model parameters which would be difficult to obtain experimentally. A species-specific model for the mouse was developed to perform simulations of dehydration and infusion based on animal experiments that were currently in

progress. At each step of the program, additional hypotheses were conceived, expressed mathematically, tested in the model against experimental data, then tested once again for their validity in space-flight simulations. The most promising hypotheses were then considered for evaluation in the laboratory.

More than in any other investigative area considered in this book, the modeling approach served to coordinate data from various ongoing ground-based experimental programs and helped maximize the utilization of the acquired information.

A REVIEW OF SPACE-FLIGHT STUDIES

The hematology experiments performed on Skylab encompassed a wide range of objectives, including assessment of man's immunological integrity, cytogenetic studies, red cell metabolism, topographical alterations of red cells, and blood volume changes. Only a small portion of these data were deemed directly applicable to describing and explaining overall disturbances in circulating red cell mass. The most relevant data obtained from human space-flight studies, including that from pre-Skylab missions, will be briefly reviewed here. This information formed the basis for developing specific hypotheses that guided the systems analysis study.

Gemini Findings

The loss of red cell mass during space flight was first observed on the Gemini 4 mission. The measured loss (12 percent in 4 days) was much higher than could be accounted for by natural attrition of cells (e.g., about one percent per day), even if production rates were completely inhibited. This suggested that increased red cell destruction was a causative factor. Subsequent studies on other flights confirmed a decrease in red cell survival (based on ^{51}Cr half-times), and suggested that a hemolytic process was responsible for the observed red cell loss. Also noted were increases in cell membrane fragility and biochemical alterations that are known to influence red cell integrity (Fischer et al., 1967).

An hypotheses was advanced that was consistent with most of these observations; it was based on the 100 percent breathable oxygen atmosphere of the Gemini space capsules. Compared to a normal earth oxygen partial pressure of 150 to 160 mmHg, the astronauts of the Gemini (and later Apollo flights) were exposed to about 260 mmHg (100 percent oxygen at 1/2 normal atmospheric

pressure). This atmosphere is known to have a toxic effect on the membranes of red cells and could have accounted for the increased destruction and reduced magnitude of the circulating red cell mass (Fischer and Kimzey, 1971).

A normal feedback compensation for destruction of cells is a compensatory increase in production rates. Reticulocyte counts are generally good indicators of bone marrow production rates. For reasons not understood at this time, compensatory erythropoiesis was not evident in these early flights, as reflected by the lack of reticulocytosis until several days after the day of recovery.

Apollo Findings

The Apollo missions were also characterized by a significant loss of red cell mass, although its severity was less than that observed during the Gemini flights. In contrast to the Gemini missions, red cell survival was not significantly altered during the inflight or postflight phases of all of the Apollo missions. This indicated that hemolysis either did not occur or was very slight (Kimzey, 1975). Like the Gemini crewmen, the Apollo astronauts breathed an atmosphere that was primarily hypobaric oxygen. However, on Apollo, the atmospheres contained small, but varying amounts of nitrogen with extended periods when nearly 100 percent oxygen was used. A qualitative analysis of all space and ground-based chamber studies performed up to that time revealed that the most severe losses in red cell mass occurred when a 100 percent oxygen atmosphere was used (compared to those situations in which a diluent gas was present (Kimzey et al. 1975)).

It was proposed that the hyperoxic atmosphere was not only capable of initiating red cell destruction, but it was also capable of inhibiting the production of red cells as well (Larkin et al., 1972). Hyperoxia is thought to limit erythropoiesis (even in atmospheres with a diluent gas) by the same feedback pathways (operating in an inverse manner) that cause enhanced production during hypoxia (Jaskunas et al., 1973). In order to account for the losses observed in some of the Apollo flights, red cell production would have to be totally inhibited for the duration of the flight, assuming a normal loss of approximately one percent per day. Taken as a whole, the data suggested that the decrements during Apollo may result primarily from diminished production of red cells rather than increased red cell destruction as was the case on Gemini.

Suppression of production on the Apollo flights was not only inferred from normal red cell survival times, but also a reduced reticulocytosis immediately upon return to earth. In light of the Apollo findings, it appears that suppressed production may have also occurred in the Gemini crew. This may be inferred from their reticulocyte counts at recovery, which, while normal, should have been much higher, based on the amount of hemolysis measured.

Table 1 shows a comparison of the percent changes in red cell mass and plasma volume in all space-flight crews in which these parameters were measured. For comparison, data for the ground control subjects are also included. Of these control studies, the subjects in all except the Skylab Metabolic Experiment Altitude Test (SMEAT) and the Brooks Air Force studies (both done in hypobaric chambers) were exposed to a normal earth atmosphere. The SMEAT study used an atmosphere (pressure and composition) similar to that of Skylab, and Brooks used an atmosphere similar to that of the Gemini atmospheres. The atmospheres of the Gemini missions were 100 percent oxygen, and the crewmen were de-nitrogenated prior to launch. The Apollo 14 to 17 flights were lunar missions, so that only partial degrees of weightlessness were encountered. No indications of hemolysis were observed on these latter Apollo flights. A significant conclusion that can be drawn from Table 1 is that there was a variable decrease in all astronauts, the severity of which was not seemingly related to the duration of flight.

The limited amount of data collected on these missions, and the complete lack of inflight observations on the indices which measured oxygen transport, red cell production, and red cell lifespan precluded establishing the exact mechanisms of the red cell mass loss. Atmospheric oxygen undoubtedly was a contributory agent, but it was probably not the only one (Kimzey, 1975). To confound the issue, bed-rest studies (used as ground-based analogs of weightless space flight) had demonstrated that significant decrements in red cell mass could occur in a normoxic terrestrial atmosphere, thereby implicating factors such as immobilization or weightless itself (Morse, 1967; Jensen, 1972). Nevertheless, the general belief was that the Skylab astronauts would be protected from red cell mass losses because the oxygen partial pressure would be much more similar to earth's atmosphere than in previous flights and because of the use of nitrogen as a diluent in concentrations higher than that used in any previous flight (Berry, 1976). The cabin atmosphere of the Skylab missions differed from that of Apollo

TABLE 1

CHANGES IN VASCULAR COMPARTMENTS DURING SPACE FLIGHT*

MISSION	DURATION (DAYS)	RED CELL MASS (% CHANGE)	PLASMA VOLUME (% CHANGE)
GEMINI 4	4	-12	- 9
GEMINI 5	8	-21	- 7
GEMINI 7	14	-14	+11
APOLLO 7-8	5-11	- 2	- 8
APOLLO 9	10	- 8	- 9
APOLLO 14-17	9-13	-10+1	- 4
SKYLAB 2	28	-14	- 8
SKYLAB 3	59	-12	-13
SKYLAB 4	84	- 7	-16
APOLLO-SOYUZ	9	- 7	-11
GROUND CONTROLS			
APOLLO 14-17**	9-13	-1.1	+10
SKYLAB 2**	28	+0.4	+ 8
SKYLAB 3**	59	+0.8	- 2
SKYLAB 4**	84	0	- 3
SHEAT	60	- 3	+ 2
BROOKS	30	-12	-
APOLLO-SOYUZ**	9	+ 1	+ 6

* Taken from Kimzey (1979)

** Subjects exposed to normal earth atmosphere

flights by having an oxygen:nitrogen ratio of 70 percent: 30 percent (at 1/3 atmospheric pressure). Therefore, it was totally unexpected when the crew of the first Skylab mission returned with red cell losses as great as those seen in the Gemini astronauts.

Skylab Findings

Although the common characteristic of all space missions has been the reduction of red cell mass and plasma volume, other hematological parameters and influencing factors did not necessarily exhibit the same uniformity, as is illustrated in Table 2. Thus, measurements of red cell mass, red cell survival, reticulocytosis, and postflight recovery differed, not only among the Gemini, Apollo, and Skylab series, but, as the following discussion reveals, among the three Skylab missions as well. The data summarized here were obtained from the published accounts of the principal investigators (Kimzey, 1975; Kimzey et al., 1976a; Kimzey, 1977; Kimzey, 1979; Johnson et al., 1977.)

Red Cell Mass and Plasma Volume:

The red cell mass losses on the 28-, 59-, and 84-day missions averaged 14.3, 12.3, and 6.8 percent respectively, as measured on the first day of recovery (see Figure 2). Although the mean change of the second flight was not statistically different from that of the first flight, the general trend would seem to imply that the red cell mass loss decreases in severity with increasing mission duration beyond one to two months, and may thereby be said to be self-limiting.

No inflight plasma volume determinations were performed. The first postflight measurements demonstrated losses of 8, 13, and 16 percent in plasma volume for the 28-, 59-, and 84-day crews, respectively, or an average loss of 360 ml for all crewmen (see Figure 2). However, plasma volume is known to change rapidly (i.e., 10 percent variations are seen during brief postural changes) and the value measured shipboard on recovery day may not reflect the true zero-g volume prior to reentry. (This is in contrast to red cell mass measurements which, in the absence of hemolysis, are relatively constant during acute plasma volume changes.)

TABLE 2
COMPARISON OF MAJOR HEMATOLOGICAL FINDINGS
ON U.S. MANNED SPACE FLIGHTS

	<u>Gemini</u>	<u>Apollo</u>	<u>Skylab</u>
100% oxygen atmosphere	Yes	No	No
Red Cell mass decrease	Yes	Yes	Yes
Plasma volume decrease	Yes	Yes	Yes
Inflight Hb increase	ND	ND	Yes
RBC survival decrease	Yes	Slight	Only 28-day mission
Reticulocytes on recovery day	No Change	Reduced	Reduced
Delayed recovery of red cell mass	Yes	Yes	Only 28-day mission
Flight Duration	4d-14d	10d-16d	28d-84-d

ND = Not Determined

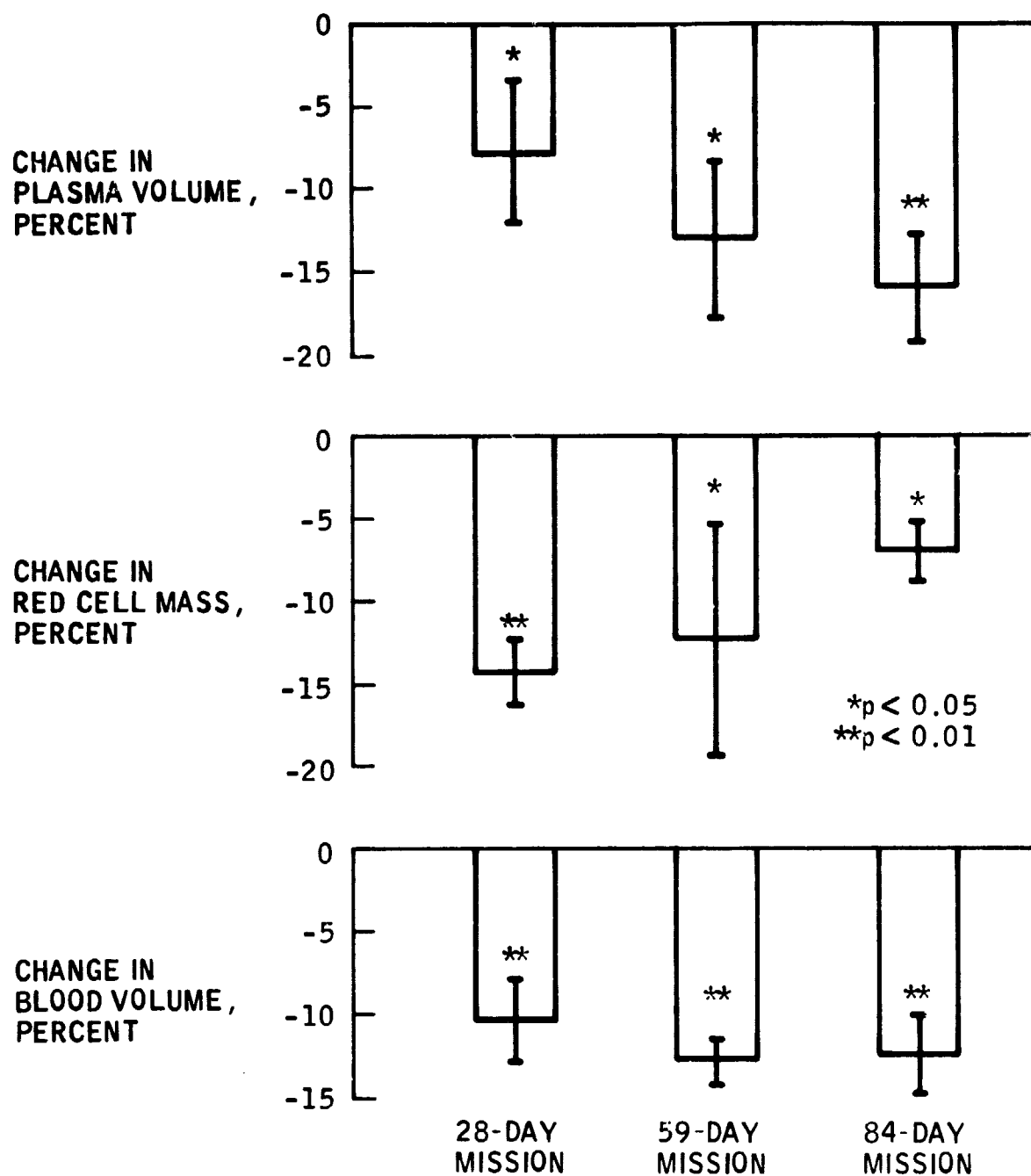


Figure 2: Changes in plasma volume, red cell mass, and total blood volume of the Skylab crew. Each bar represents the mean (+SD) difference between preflight and postflight measurements for each three-man mission. The postflight value was obtained on the day of recovery.

Hemoglobin Concentration:

Hemoglobin concentrations were measured inflight using a hemoglobinometer in conjunction with each inflight venipuncture. Postflight, hemoglobin measurements of inflight blood were made by biochemical analysis of the returned frozen samples (Kimzey et al., 1976a). Inflight data were obtained by these two procedures only on the crews of the 59- and 84-day missions so that hemoglobin alterations for the 28-day mission are not known. The precision of the inflight data is admittedly less than that associated with pre- and postflight determinations.*

Consistent in all the inflight Hb determinations was an elevated value in the first inflight sample, presumably due to a loss of plasma volume. If hemoglobin concentration disturbances are used as an index of plasma volume changes (Van Beaumont et al., 1972), it would appear that the rate of change of plasma volume during space flight is extremely rapid. The earliest Hb measurement was obtained on the third inflight day, and showed an average (N=6) increase of about 11 percent. Assuming red cell mass did not change during this time, this translates into a plasma volume decrease of approximately 500 ml, or 18 percent below control. It is not valid to use hemoglobin measurements after this point to estimate plasma volume, since red cell mass is also changing at an unknown rate.

As the mission progressed, Hb concentrations remained elevated, but there was a gradual reduction in their value toward normal. The latter trend would indicate a gradual drop in red cell mass if plasma volumes were stable at a reduced level. However, plasma volume determinations were not performed inflight. Figures 3, 4, and 5 summarize all the known Skylab data with respect to Hb concentrations, red cell mass, and plasma volume.

* Hb concentration values were higher when measured inflight, compared to the postflight measurements on samples obtained inflight. This difference was noted (Kimzey, 1977), but no cause could be found. However, it is possible that the somewhat hyperoxic atmosphere (190 mmHg oxygen partial pressure) may have been responsible for increasing oxygen saturation of blood samples measured in vitro by the hemoglobinometer technique.

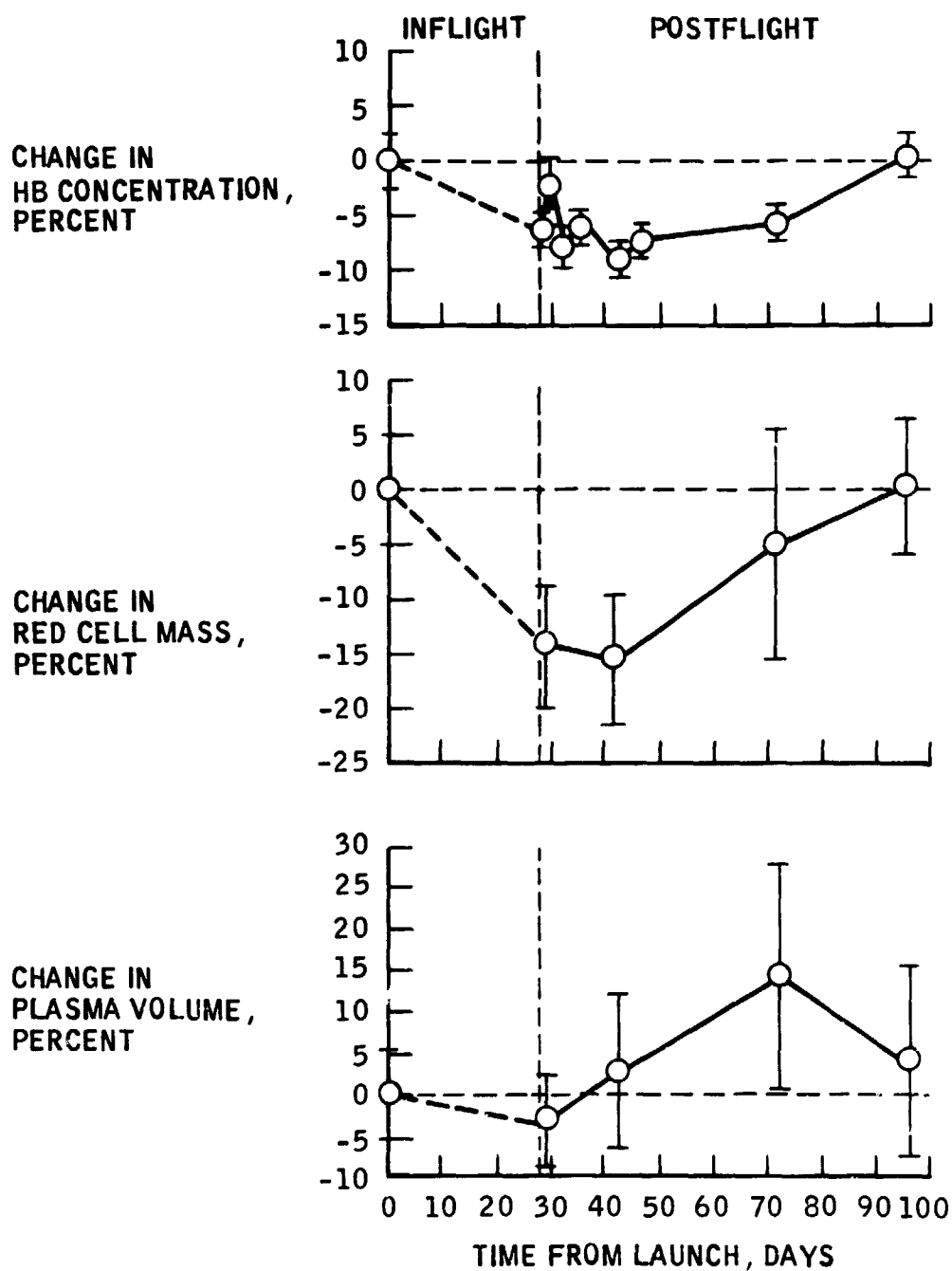


Figure 3: Hematological parameters measured as a function of time from launch for the inflight and postflight phases of the 28-day mission. Mean (\pm SD) values for the three crewmen are expressed as percent change from preflight control levels. No inflight measurements were obtained for red cell mass and plasma volume. Plasma hemoglobin was not measured during the inflight phase on the 28-day mission.

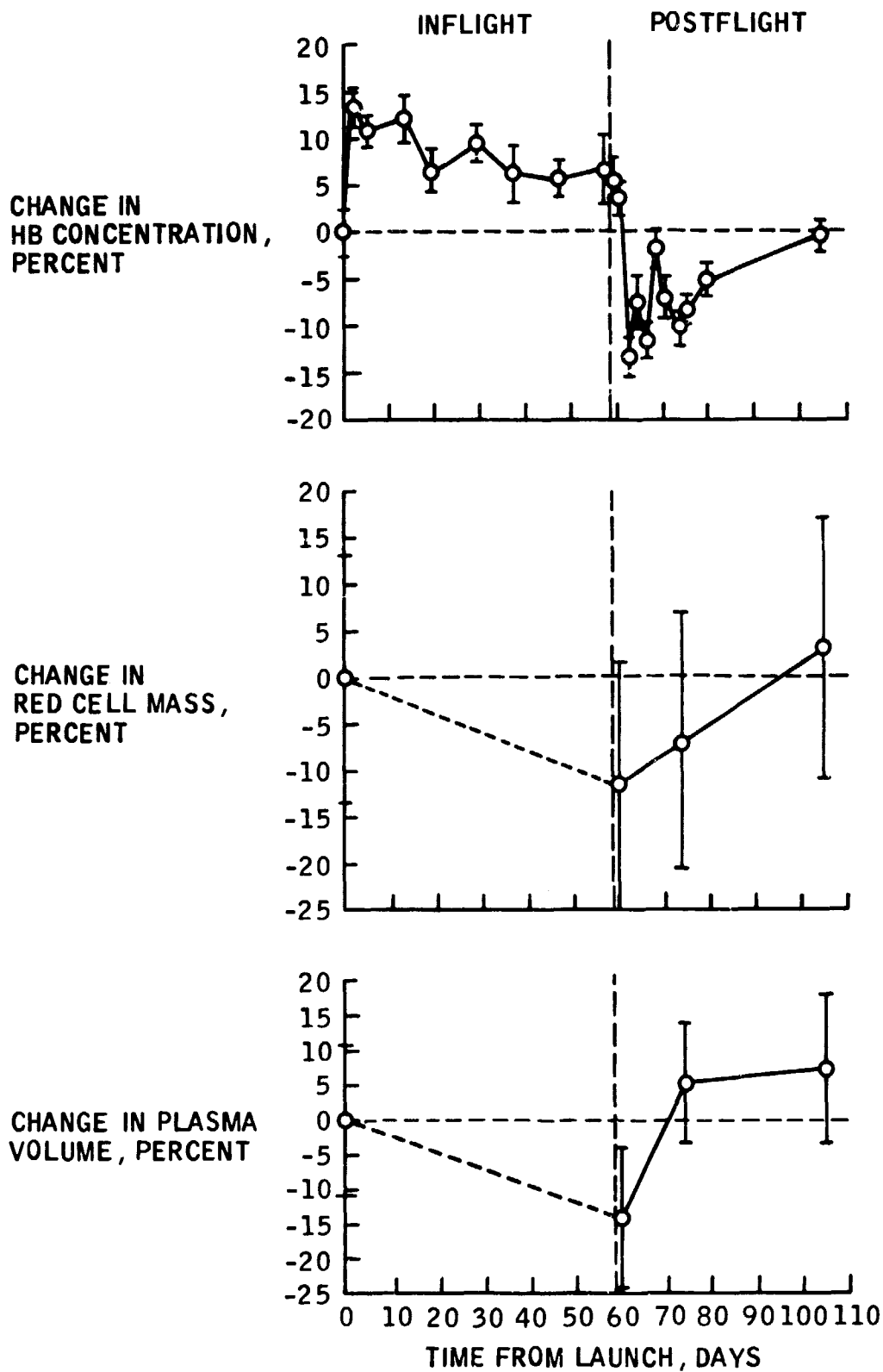


Figure 4: Hematological parameters measured as a function of time from launch for the inflight and postflight phases of the 59-day mission. See caption in Figure 3.

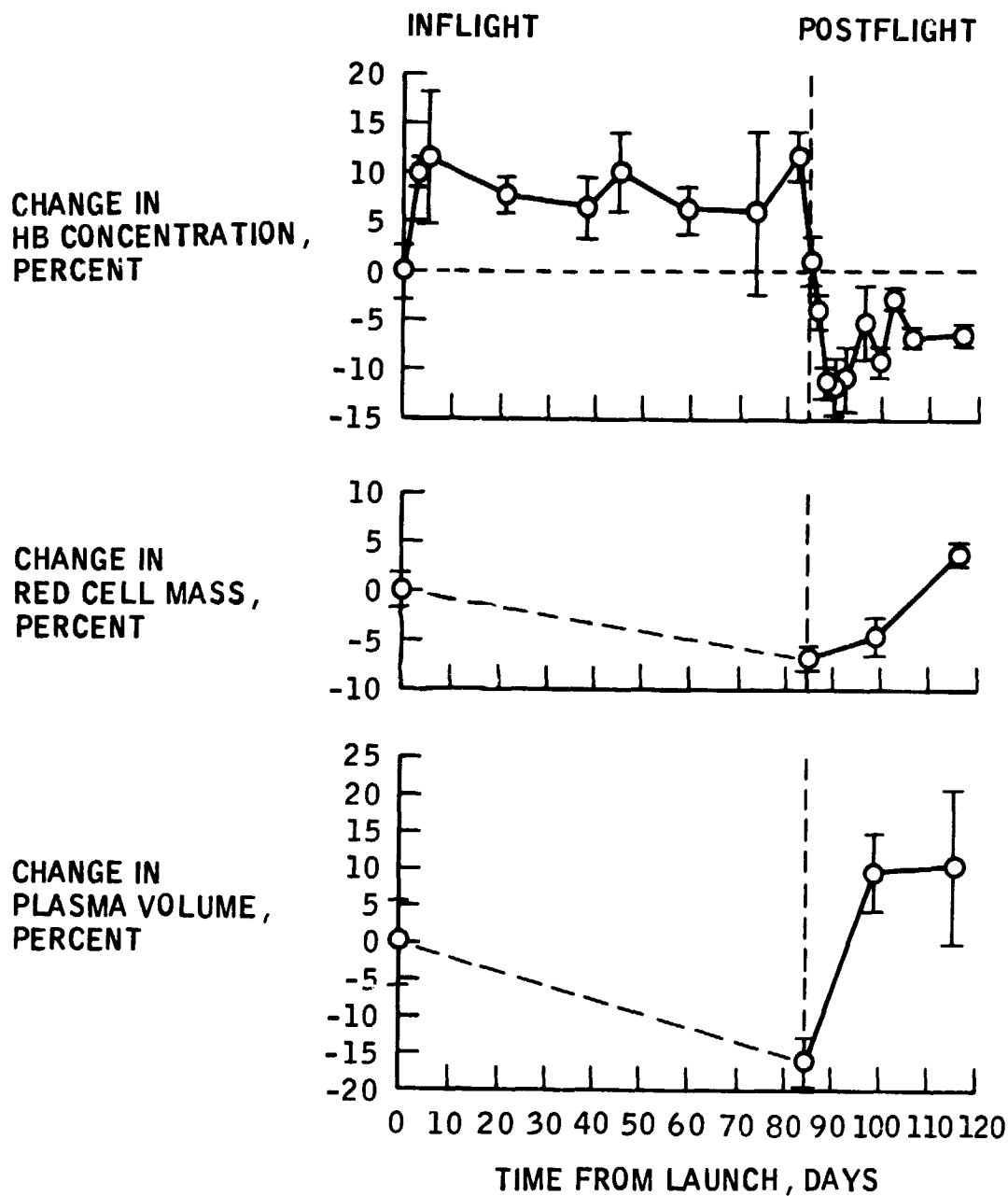


Figure 5: Hematological parameters measured as a function of time from launch for the inflight and postflight phases of the 84-day mission. See caption in Figure 3.

Lifespan Studies:

Indicators of intravascular hemolysis were provided by both ^{51}Cr red cell halftimes and ^{14}C -glycine red cell mean lifespans. Treating the entire population of nine crewmembers as a single group, there were no statistically significant differences between the preflight and postflight mean values, or between the flight crew and one-g control group for either of the two measurement methods (Johnson et al., 1977). However, the postflight ^{51}Cr halftimes for the crewmen of the 28-day mission were 18 percent less than their preflight values ($p < 0.05$). If their ground control effects are removed, the net effect due to space flight is a 12 percent decrease ($p < 0.05$) in red cell halftime (see Table 3). This indicates that hemolysis may have been a factor on that mission. The red cell mass loss of this particular flight was also the largest of the three missions.

Reticulocyte Counts:

Postflight reticulocyte counts from all three missions are shown in Table 4. Increases in reticulocytes can be taken as an index of red cell production. The low values on the first recovery day suggest suppressed red cell production, and imply that this is a carry-over from the previous days spent in zero-g. The general tendency of reticulocyte counts to rise toward and above normal during the following weeks indicates augmented production and regeneration of red cell mass. The differences between the first crew and the last two are noteworthy. The postflight reticulocyte counts of the crew of the 28-day mission rose much more slowly than those of the two longer missions, and they never rose above preflight values. Also, each subsequent crew exhibited higher reticulocyte counts on the first day of recovery and the counts rose fastest for crews that remained in space longer. These results are generally consistent with the pattern of inflight red cell mass loss and postflight recovery reflecting zero-g suppression followed by enhanced production of erythrocytes.

Change in Red Cell Shape:

Special hematological studies of the inflight blood samples showed that during extended exposure to space flight, significant alterations occur in the distribution of red cell shapes in the peripheral circulation (Kimzey, 1975; Kimzey, 1977). Severe deformation of circulating red cells can result in

TABLE 3

Percent Change in ^{51}Cr Red Cell Half-Times of Skylab
Crewmembers and Control Subjects (Johnson et al., 1977)

<u>Mission</u>	<u>N</u>	<u>Skylab Crewmembers</u>	<u>Ground Controls</u>	<u>Differences Due To Space Flight</u>
28-day	3	-17.8 \pm 5.0* ⁺	-6.0 \pm 3.6	(p<0.05)
59-day	3	- 6.6 \pm 1.1*	-5.9 \pm 9.8	(NS)
84-day	3	+ 4.3 \pm 10.3	-3.5 \pm 4.2	(NS)
Skylab Mean	9	- 7.3 \pm 11.1	-5.4 \pm 6.4	(NS)

* Different from preflight (p<.05)

+ Different from control group (p<.05)

NS Not Significant

TABLE 4

SKYLAB CREWS RETICULOCYTE COUNTS
(PERCENTAGE OF PREMISSION MEAN)

<u>MISSION</u>			
DAY	28-DAY MISSION	59-DAY MISSION	84-DAY MISSION
R + 0	44	69	88
R + 1	53	--	117
R + 3	67	87	87
R + 7	80	184	143
R + 14	86	227	184
R + 21	93	234	180

their premature sequestration by the reticuloendothelial system (RES). The alteration in red cell shape during space flight might provide a sufficient stimulus to the RES to initiate trapping and eventual removal of these cells from the circulating red cell mass. Maintenance of normal red cell shape and normal deformability are essential to survival of the cell in vivo. A major function of the RES is to remove from circulation those cells whose structure is abnormal or whose membrane is too rigid. It was not possible to substantiate a direct relationship between the red cell shape alterations during the Skylab missions and the concomitant loss in red cell mass, but this is an area that merits further investigation (Kimzey, 1977).

Bed-Rest Findings:

Experimental bed rest involving healthy subjects has been considered an analogous stress to weightlessness because it minimizes certain effects of gravity, particularly hydrostatic and load-bearing effects in the longitudinal direction. The earliest of these studies (Taylor et al., 1945), as well as those conducted very recently (Johnson and Driscoll, 1977; also see Figure 6) have invariably reported blood volume decrements both in the plasma and red cell components. Typically, for studies lasting less than one month, red cell mass losses are greater during space flight and plasma volume losses are greater during bed rest (Johnson, 1979).

Unlike space flight, the time-varying behavior of these blood volume components is more easily examined during bed rest. Figure 6 illustrates several responses which are typical of many bed-rest studies. As shown in the figure, red cell mass declines linearly as a function of time (Johnson and Driscoll, 1977), while plasma volume declines in roughly exponential fashion with half the total loss occurring the first few days and the remainder lost over several weeks (Johnson et al., 1971; Greenleaf et al., 1977). Hematocrit and hemoglobin concentration increases within several days by 5-10 percent and remains elevated despite the loss in red cell mass because plasma volume is also disappearing from the circulation (Morse, 1967). Following bed rest, the plasma volume increases rapidly (resulting in hemodilution), but red cell mass does not begin to regenerate until after one or two weeks (Leonard, 1977).

The fact that these changes are qualitatively similar to those which are found after space flight suggests that similar mechanisms may be operative in these two hypogravic stresses. The validity of this assumption has not been

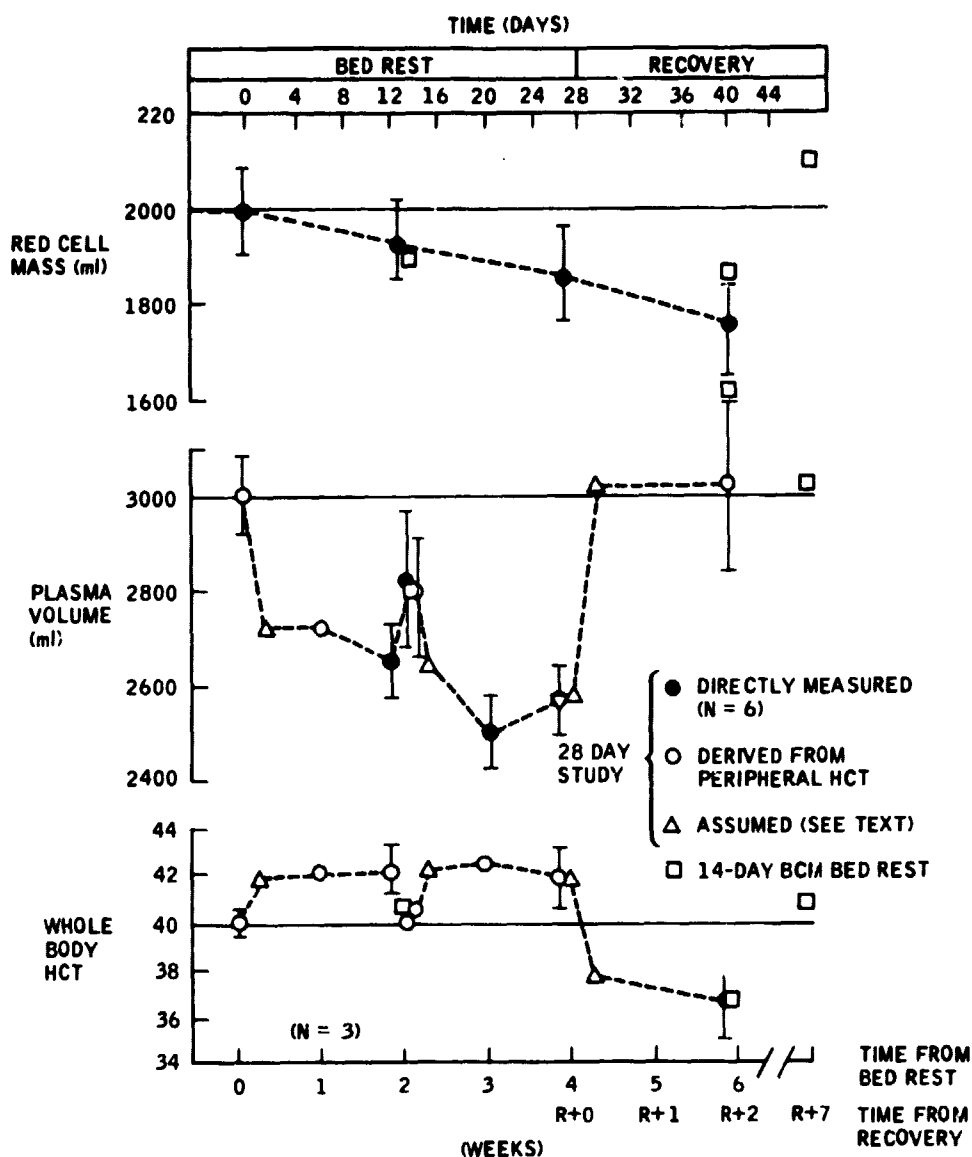


Figure 6: Hematological measurements from a two-week and four-week bed-rest study (Johnson and Driscoll, 1975; 1977). The data are normalized with respect to the computer model's control values (indicated by the solid horizontal lines). The symbols designated as "assumed" provide the most realistic estimates of plasma volume where large fluid shifts are believed to occur, but were not measured. These assumptions were necessary to obtain accurate simulations with the computer model (see subsection "Bed-Rest Simulations" later in this section). The rationale for these assumptions is available elsewhere (Kimzey et al., 1979). During days 13, 14, and 15, each subject received a one-liter saline solution administered orally as part of an experiment to test its efficacy against orthostatic intolerance.

firmly established. Unfortunately, most bed-rest studies have not been directed toward examining the erythropoietic system in detail. Evidence for suppression of erythropoietic activity is somewhat stronger for bed rest than it is for space flight, although indications of increased cell destruction have also been reported (Morse, 1967). Reductions in reticulocyte counts and erythropoietin levels have been observed (Morse, 1967; Shcherba et al., 1975) but not consistently (Dunn et al., 1977).

Taken together, these data indicate erythro-suppression, possibly mediated by an excess of oxygen supply over demand. However, the hypo-metabolism of bed-rest subjects in comparison to the more normal metabolic activity of the Skylab subjects makes comparison of these stresses difficult. Likewise, the normoxic environment in which bed rest is usually studied is not comparable to the hyperoxic atmosphere of the earlier Gemini and Apollo missions. In addition, losses of red cell mass have only been measured for up to 35 days during bed rest, which provides few clues to the regenerative behavior suggested in space flights beyond 60 days. These considerations were examined systematically in the study described here.

Preliminary Hypotheses:

A summary of the major space-flight findings related to red cell loss are shown in Table 5. Any interpretation of these observations should be consistent with the fact that, qualitatively and quantitatively, the first Skylab mission had a somewhat different response than the two longer missions (see Table 6). In their preliminary Skylab reports, Johnson et al. (1977), Kimzey (1977), and Dietlein (1977) offered several hypotheses to explain the hematological findings; these are summarized in Table 7 and below.

The etiology of the drop in red cell mass and lowered reticulocyte counts at recovery is unknown. The red cell mass is the most stable of the various blood constituents, and rapid changes are only possible in hemorrhaging or hemolysis. Gradual decreases may be produced by inhibition of bone marrow activity, ineffective erythropoiesis, hemolysis, chronic hemorrhage, or sequestration of cells. The space-flight experiments were sufficiently comprehensive to rule out most of these factors. There was no clinical indication of hemorrhage among the crews, and data collected during the mission did not support the concept of intravascular hemolysis as reflected by normal ^{51}Cr halftimes, normal ^{14}C -glycine lifespans, and normal haptoglobins

TABLE 5

SUMMARY OF SPACE-FLIGHT FINDINGS

- o Red cell mass consistently reduced during space flight
- o Evidence of hemolysis on Gemini flights
- o No consistent evidence of hemolysis on Apollo and Skylab flights
- o Rate of red cell loss in Skylab greater than observed during bed rest studies of comparable duration
- o Reticulocytes reduced immediately after recovery
- o Postflight reticulocytosis is often delayed several days
- o Hemoconcentration occurs early in space flight
- o Hemodilution occurs during postflight period
- o Plasma volume reduction measured postflight
- o Significant alterations in red cell shape
- o Hematological responses of the first Skylab mission different than for second and third missions (see Table 6)

TABLE 6

OBSERVATIONS FROM THE 28-DAY SKYLAB FLIGHT THAT DIFFERED
FROM THE 59-DAY AND 84-DAY MISSIONS

1. Red cell loss was the greatest of the three Skylab missions
2. Rate of postflight recovery of RCM was delayed until after two weeks from reentry; other Skylab crews did not show delay
3. Only crew in which reticulocytosis did not occur postflight
4. Plasma volume losses were the least as measured postflight
5. Only crew to have statistically different ^{51}Cr halftimes
6. Hematocrits and hemoglobin decreased during postflight period (resulting from plasma refilling) as on other flights, but was decreased the least (half as much) on the 28-day flight
7. Diet and exercise were the least adequate
8. Crew lost the most weight, suggestive of negative energy balance

TABLE 7

PRELIMINARY HYPOTHESES TO EXPLAIN SPACE-FLIGHT HEMATOLOGICAL FINDINGS

- o Erythropoiesis is suppressed during exposure to weightlessness
- o Red cell destruction contributed to Gemini red cell losses and possibly to a smaller extent during Apollo, while red cell lifespan was normal on the average in Skylab
- o Hyperoxic atmosphere contributory to red cell loss in Gemini and Apollo
- o Red cell mass loss is self-limiting and regenerative
- o Kinetics of red cell mass recovery (postflight) independent of duration of weightlessness exposure
- o Hemoconcentration during space flight and bed rest a result of rapid and sustained reduction in plasma volume
- o Hemodilution during postflight phase a result of plasma volume rapidly returning to normal
- o Red cell mass loss may be due to splenic trapping of red cells promoted by alterations in red cell shape
- o Rule out hemolysis, splenic trapping of red cells, ineffective erythropoiesis, blood sampling effects, hemorrhage
- o Hemoconcentration and increased affinity contributed to inadequate erythropoietic response to the loss of red blood cells during the first month

(Johnson et al., 1977). Iron kinetic studies (serum iron, iron turnover, and iron reappearance) tended to rule out ineffective erythropoiesis (Kimzey, 1975), a conclusion also reached in bed-rest studies (Johnson and Driscoll, 1977). The low reticulocyte counts at recovery are additional evidence against ineffective erythropoiesis. A proposed splenic trapping of circulating cells (Johnson et al., 1977) was unconfirmed by spleen and liver scans during the Apollo-Soyuz flight, the only U.S. manned space mission to be performed after Skylab (Kimzey and Johnson, 1977) for a number of years. Finally, the control subjects showed little change in either red cell mass or reticulocyte counts, ruling out effects resulting from the blood drawing schedule. Taken as a whole, these data suggest, by inference, that the Skylab red cell mass losses were a result of decreased production rather than an increase in red cell destruction.

The early published accounts of the Skylab experiments left the etiology of the probable decrease in bone marrow activity unanswered. Johnson et al. (1977) addressed the question of why the bone marrow failed to replete red cells in space flight. They proposed that hemoconcentration or rightward shifts in the oxy-hemoglobin equilibrium curve may have maintained oxygen flow to the kidney and prevented erythropoietin secretion.

Whatever the cause of the decreased red cell mass, it was suggested that the losses were self-limiting, since increased time spent in space did not result in additional loss. Furthermore, the smaller losses of red cell mass on each longer mission gave rise to a "regeneration" theory. Accordingly, red cell mass initially decreases during the first 30 days of flight and this is followed by a gradual recovery of red cells which begins approximately 60 days after launch (Johnson et al., 1974; Dietlein, 1977; Kimzey, 1977). Although no measurements of red cell mass were made inflight, this theory has a basis of support from a composite time profile of the postflight red cell mass measurements (Figure 7).

The primary objective of the hematology systems analysis was to assist in the interpretation of the Skylab findings. More specifically, it was designed to develop and test hypotheses, using computer simulation techniques, to explain the loss of red cell mass during space flight, and the self-limiting nature of the Skylab crew's red cell mass loss, as well as the difference in recovery kinetics between the first flight and the last two flights.

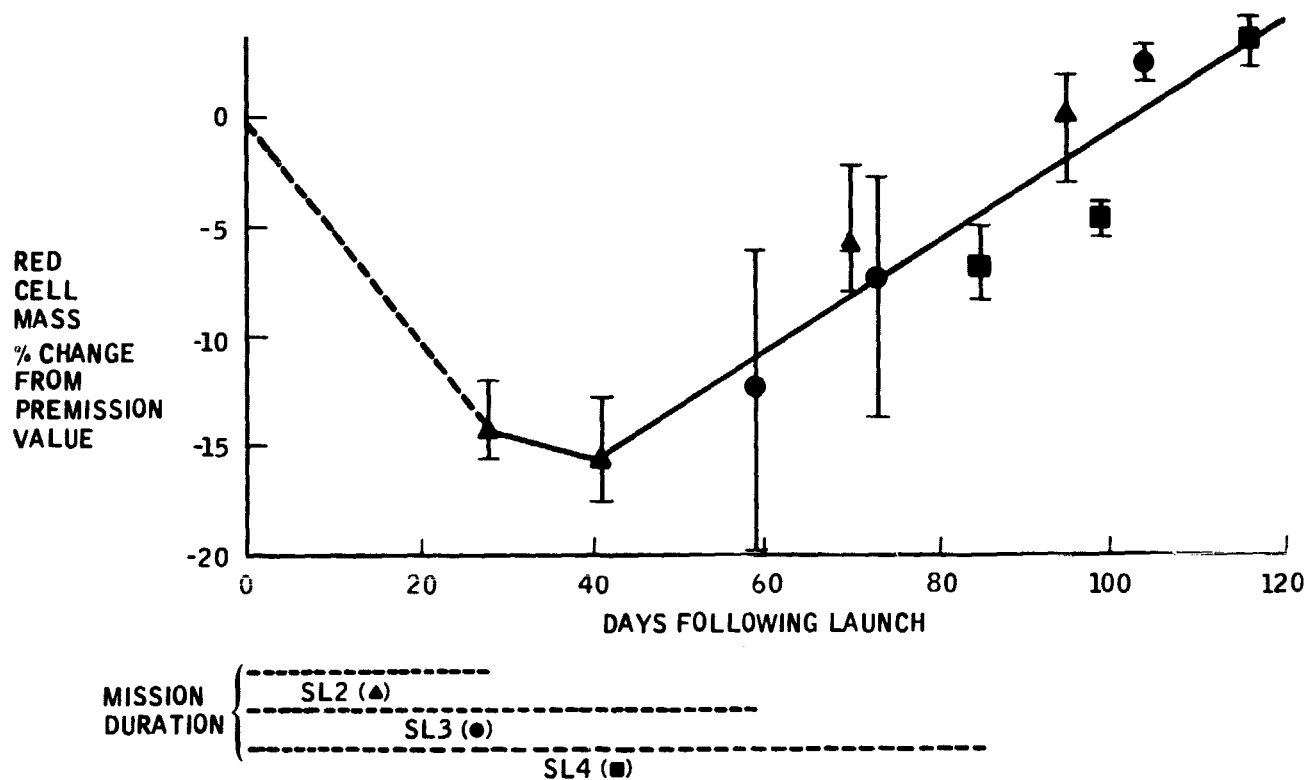


Figure 7: Changes in red cell mass in the Skylab crew measured from the day of launch. Each point represents the mean (+SD) changes of the three-man crews as measured on subsequent days of the postflight period. The first measurement for each crew was obtained on the day of recovery. The solid line through the points (calculated by least squares method) support the hypothesis that regeneration of red cell mass begins after 40 days from launch. (Figure obtained from Kimzey, 1977).

Ground-based studies, including those specifically sponsored by NASA, were performed on human and animal subjects and carried out with the view of explaining these findings on an experimental basis. An additional objective of the present analysis was to interact with the experimental investigators by suggesting experimental objectives assisting in data interpretation, and using the newly acquired data to modify the model, if necessary.

THEORETICAL CONSIDERATIONS OF ERYTHROPOIESIS: TISSUE OXYGENATION

An important assumption in this study was that the basic interrelationship between tissue oxygenation and erythropoiesis would provide clues for understanding the zero-g loss of red cell mass. This assumption was derived from the accepted physiological concept that the balance between oxygen supply and demand at the tissue level is the major determinant of bone marrow erythropoiesis. Some of the important considerations regarding tissue oxygenation will be reviewed here to provide a theoretical foundation for developing specific hypotheses relevant to the space-flight findings. A mathematical model embodying the essential elements of tissue oxygenation and erythrocyte production is also summarized. This model, especially its physiological basis and its simulated behavior, was the basis for interpreting the space-flight events.

Physiology of Tissue Oxygenation

An adequate supply of oxygen to individual tissues is dependent on a circulatory system which transports oxygen, bound reversibly to hemoglobin, from an oxygen loading organ to the capillaries of an oxygen consuming organ. Oxygen enters the cellular space by diffusion along an oxygen tension gradient between the capillary and the cell. Normally, a steady-state exists between the rate of oxygen consumed by the tissues and the rate of oxygen delivered to the tissues. The amount of oxygen delivered to a tissue depends on a number of factors, the most obvious of which are oxygen tension of inspired air, pulmonary function, hemoglobin concentration (Hb), affinity of hemoglobin for oxygen, speed of dissociation of oxygen from hemoglobin, cardiac output, and the vascular distribution of circulating blood among the various tissue (Harris and Kellermeyer, 1970; Finch and Lenfant, 1972). A disturbance in any of these factors, or in the rate of metabolism, will cause a temporary imbalance between tissue oxygen supply and demand and, thereby, will change tissue oxygenation.

At the tissue level, the rate of arterial oxygen supply can be expressed as follows

$$\begin{aligned} \text{Oxygen flow rate} = & \text{blood flow rate to tissue} \\ & \times \text{fraction of Hb saturated with oxygen} \\ & \times \text{Hb concentration in blood} \\ & \times \text{O}_2 \text{ carrying capacity of Hb (normally constant)} \end{aligned}$$

Of these factors, the bone marrow elements of the erythropoiesis system primarily regulates only one, the hemoglobin concentration. Control of blood flow and hemoglobin saturation may be considered to be under the domain of the circulatory, respiratory, and blood biochemical regulatory systems (see Figure 8). These latter systems are capable of rapid compensatory adjustments in the face of small changes in the oxygen supply-demand balance. For example, changes in the number of active tissue capillaries and blood flow adjustments are common circulatory responses to minor local fluctuations in the degree of tissue oxygenation. Under these conditions, the rate of red cell production and the circulating red cell mass are essentially invariant. However, in more extreme and chronic situations such as hemorrhage, altitude hypoxia, or pulmonary disorders, an increase in red cell production and circulating red cell mass appears to be a major pathway by which the body compensates for an insufficient tissue oxygen supply (Hannon and Vogel, 1977). Similarly, conditions that lead to chronic levels of tissue hyperoxia, such as breathing from an hyperoxic atmosphere, red cell transfusion-induced polycythemia, or when the tissue demand for oxygen is decreased as in hypothyroidism, hypophysectomy, and starvation, there is a significant decrease in erythropoiesis (Krantz and Jacobson, 1970).

In assessing the etiology of the loss of red cells during space flight, it is plausible to look for factors that chronically disturb the delicate balance between tissue oxygen supply and demand. Since short-term alterations in tissue oxygenation can theoretically be corrected by the circulatory, respiratory and biochemical systems, it is likely that disturbances in the erythropoiesis system itself are responsible for the longer-term space-flight findings. However, the behavior of the erythropoietic processes must ultimately be judged in the context of the total oxygen transport system.

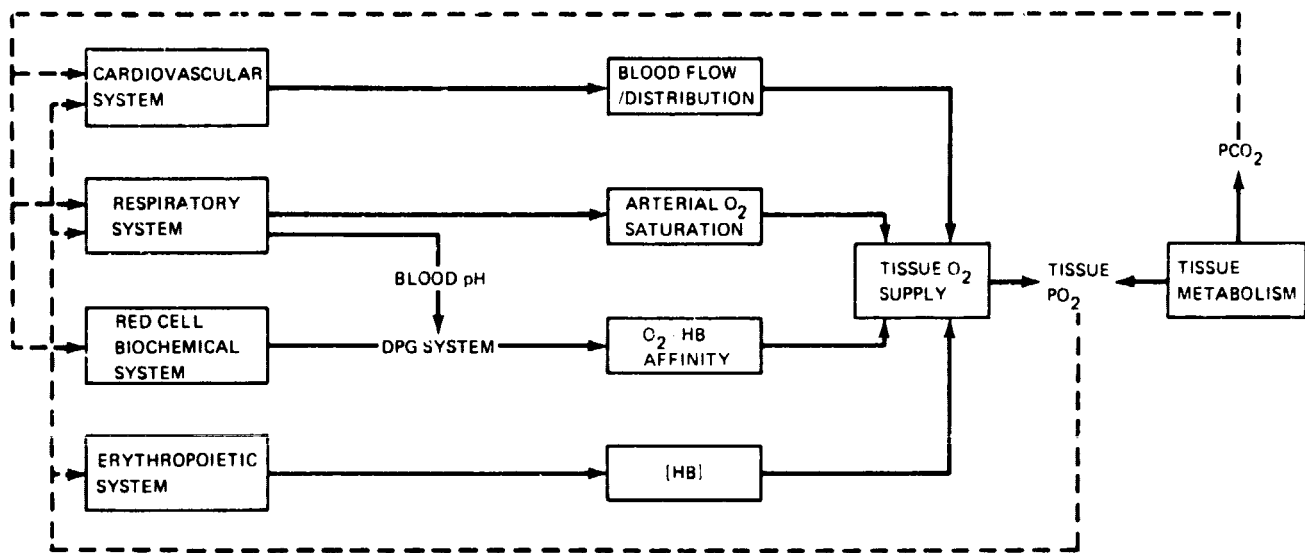


Figure 8: Regulation of tissue oxygen tension showing the contributions of the cardiovascular, respiratory, biochemical, and erythropoietic systems.

Concept of a Renal pO_2 Sensor

A majority of experimental evidence supports the hypothesis that oxygen supply to a renal "detector" site in relation to the oxygen demand of that region is the primary stimulus for erythrocyte production via the release of a renal erythropoietic hormone, erythropoietin (Krantz and Jacobson, 1970; Harris and Kellermeyer, 1970; Wintrobe, 1973). Erythropoietin production is believed to be dependent on renal tissue oxygen tension, a variable reflecting the summation of renal oxygen supply and oxygen consumption.

The location of the renal sensing site for monitoring oxygen tension and releasing erythropoietin has still not been isolated. However, anatomical and physiological considerations suggest that the kidney tissue has developed a specialization that enables it to function as a sensitive oxygen chemoreceptor. Several important characteristics of the sensing site have been identified, as follows:

First, while most organs in the body exhibit a blood distribution pattern that results in an even release of blood oxygen, the kidney has a peculiar microcirculation that favors a reduced hematocrit in the smaller vessels and a steep gradient of tissue oxygen tension along the cortico-medullary axis (Krantz and Jacobson, 1970; Gordon and Zanjani, 1970). Thus, a relative hypoxia is present which may act as a continual stimulus for daily erythropoietin production.

Secondly, the kidney, and especially the renal cortex, has a uniquely low arteriovenous oxygen difference. Its capillaries differ from the rest of the capillaries of the body in that they lie along the flat upper part of the blood oxygen dissociation curve, where small changes in oxygen concentration are associated with large changes in pO_2 . This characteristic amplifies the signal for erythropoietin production (Metcalf and Dhindsa, 1972; Selkurt, 1963).

Thirdly, the sensors which regulate erythropoietin production appear to monitor venous or tissue pO_2 , rather than arterial pO_2 (Hodgson, 1970; Adamson et al., 1969; Weil et al., 1968).

Fourthly, evidence suggests that the sensing sites are located in areas where blood flow and oxygen consumption are held stable over a wide range of oxygen tensions (Aperia et al., 1968). It is well known that autoregulation of blood flow is a specialized feature of the kidney (Selkurt, 1960). In

addition, changes in blood flow, if they did occur, would be expected to have a dampened effect on tissue pO_2 because of the unique coupling in the kidney of blood flow and oxygen consumption (Pitts, 1968).

These characteristics effectively make these renal sites a sensor of blood hemoglobin levels (or hematocrit)* for the following reasons. Since oxygen delivery to tissue is dependent essentially on blood flow rate, oxygen hemoglobin affinity, and hemoglobin concentration (see equation in previous subsection), a system that can keep blood flow and oxyhemoglobin affinity constant will deliver oxygen at a rate proportional only to hemoglobin concentration. In addition, by requiring a constant rate of oxygen consumption, the tissue pO_2 will vary in direct proportion to hemoglobin concentration.

Therefore, the renal oxygen sensor can be construed, under the conditions specified above, as a highly sensitive, high gain hemoglobinometer of the body (Beutler, 1969). When combined with a controller that can vary the rate of hemoglobin production, this system may be capable of regulating hemoglobin levels and tissue oxygen tension. This view of the erythropoiesis controller has not been fully recognized in the past and may provide important clues to bed-rest and space-flight hematological changes, inasmuch as these stresses are often accompanied by alterations in hematocrit.

Effect of Hematocrit Levels on Oxygen Transport

The influence of plasma hemoglobin concentration on oxygen delivery to the tissues, discussed in the preceding paragraphs, is more complex than the simple linear effect suggested by the oxygen flow rate equation. This is true because two of the factors that determine blood oxygen delivery (blood flow and hematocrit) have an interdependent relationship. Blood viscosity increases as the proportion of red cells in blood increases; also, blood flow is inversely related to blood viscosity (Guyton et al., 1973). Thus, a changing hematocrit can have two opposing effects on oxygen transport. First, as the hematocrit is increased, the oxygen content of the blood is increased. Second, as the hematocrit is increased, the rate of blood flow to the tissues is decreased because of the increased blood viscosity.

* Hematocrit is directly related to the blood Hb concentration by the mean corpuscular Hb concentration, MCHC, which is normally quite constant.

Therefore, it is not apparent in this situation whether there will be an actual improvement in oxygen supply to the tissues. In fact, these opposing effects would be expected to produce a maximum level of oxygen transport at a specific hematocrit (i.e., the "optimal" hematocrit). Oxygen transport would be reduced below the maximum level if hematocrit were reduced (i.e., oxygen concentration effect) or if hematocrits were increased (i.e., viscosity-flow effect) away from the optimum. The concept of an "optimum hematocrit" for oxygen transport is supported by studies of experimentally-induced anemia and polycythemia in both normovolemic and hypervolemic animals and in man (Murray et al., 1969; Replogle and Merrill, 1970).

These relationships are shown graphically in Figure 9. The influence of hematocrit on blood oxygen concentration and on blood flow are shown in Figure 9a. Both of these relationships are essentially linear over a wide hematocrit range. Oxygen transport, shown in Figure 9b is taken as the product of blood flow and oxygen concentration; i.e., the product of the two linear relationships in Figure 9a. The inverse linear relationship between blood flow and hematocrit has been cited on numerous occasions (Richardson and Guyton, 1954; Fowler and Holmes, 1975; Weisse et al., 1966; Replogle and Merrill, 1970; and Thorling and Erslev, 1968). Thus, blood with a high hematocrit may have a rheologic disadvantage with respect to oxygen transport in spite of the advantages of an increased oxygen capacity.

An additional effect shown in Figure 9 is that of blood volume on blood flow (dashed lines). One effect of an increased blood volume is a decrease in geometric vascular resistance (passive distension of blood vessels), and an increase in cardiac output at any given hematocrit level. The relationships for normo- and hypervolemia are shown in Figure 9 as solid and dashed lines, respectively. The family of parabolic shaped curves, shown in Figure 9b, representing the effects of blood volume, theoretically shifts the optimum hematocrit to higher levels as blood volume increases. (Hypovolemia will cause a shift to a lower optimum hematocrit.) More important, however, is the fact that hematocrits higher than the normal optimum do not necessarily result in reduced oxygen transport when accompanied by hypervolemia. This upward shift of the oxygen transport-hematocrit relationship helps explain why, in cases of acute hypertransfusion or chronic cases of polycythemia vera (i.e., expanded red cell mass and blood volume), oxygen transport is not depressed, but may in fact be enhanced (Castle and Jandl, 1966; Thorling and Erslev,

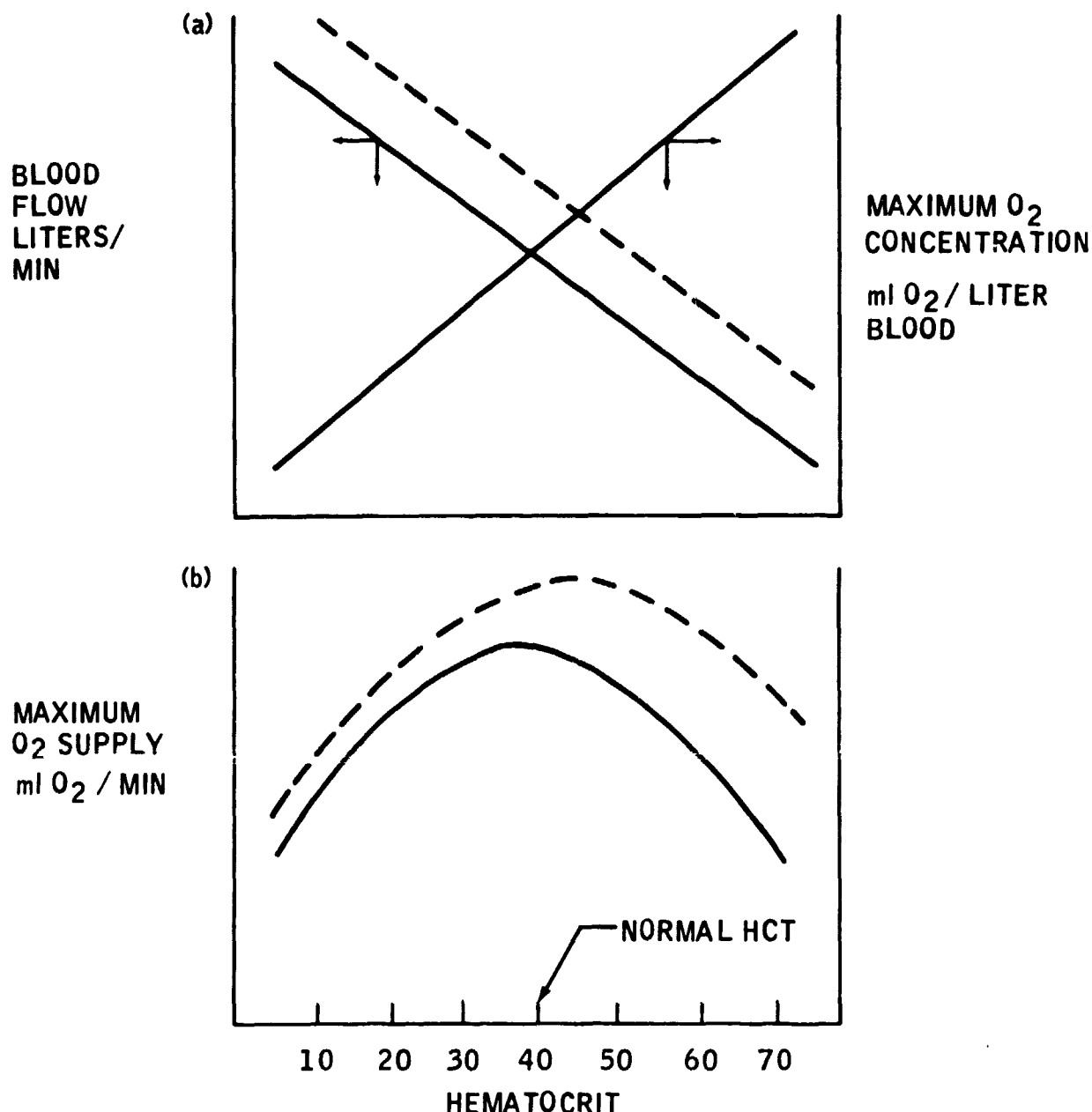


Figure 9: Effect of hematocrit on oxygen transport: (a) maximum blood oxygen concentration varies directly and blood flow varies inversely with hematocrit, (b) maximum oxygen transport, calculated as the product of blood flow and maximum oxygen concentration, is a parabolic shaped curve when related to hematocrit, with an optimum occurring in the hematocrit range for humans. As hematocrit increases, the maximum amount of oxygen that can be transported to the tissues also increases initially, but declines at very high hematocrits due to the effects of viscous flow resistance. The solid line indicates normovolemia; hypervolemia, indicated by the dashed line, causes the optimum levels to shift as shown. (Redrawn from Guyton, Jones, and Coleman, 1973; Castle and Jandl, 1966).

1968). Also, some important differences between the hemoconcentrating stresses of either an absolute erythrocytosis (i.e., red cell infusion) and a relative erythrocytosis (i.e., dehydration) are more readily appreciated in light of this analysis (see "Dehydrated Mouse Simulations" and Figure 32).

The preceding discussion serves to emphasize that predicting the level of oxygen transport requires consideration of the independent and opposite influences of blood viscosity and blood volume on blood flow. During exposure to weightlessness and in ground-based studies related to space-flight hematology (i.e., bed rest, dehydration, and infusions), both the viscosity and volume of blood are known to change to modest extents. With regard to erythropoiesis regulation, any disturbance of oxygen transport needs to be examined in the region of the renal oxygen sensors. These tissues, which release the erythrocyte-stimulating factor, are a crucial part of the feedback control system that corrects long-term changes in oxygen transport. Unfortunately, the effects of viscous resistance and passive distention on renal blood flow are not well established, particularly for this organ, which has unique flow autoregulatory capabilities. Nevertheless, the relationship between the factors determining oxygen transport, exemplified in part by the oxygen flow rate equation and Figure 9, have contributed to formulating a consistent and unified interpretation of many diverse studies, both in human and in animal subjects.

Tissue Oxygenation and Erythropoiesis: A Model

The physiologic role of the erythron is to provide an adequate amount of available oxygen in arterial blood at a suitable oxygen tension. Therefore, a complete characterization of erythropoiesis must consider the relationship between tissue oxygenation and the red cell producing mechanisms. It is generally accepted that this relationship can be described in terms of a negative feedback control circuit. Figure 10 illustrates the essential physiological and anatomical elements of such a control system, showing the relationship between blood oxygenation, tissue oxygenation, erythropoietin, red cell production, and hemoglobin concentration.

The concept shown in Figure 10 was translated into a mathematical model for the purposes of quantitatively simulating the responses to erythropoietic disturbances. This model, including its mathematical description and validation, has been fully described elsewhere (Leonard et al., 1981), as well

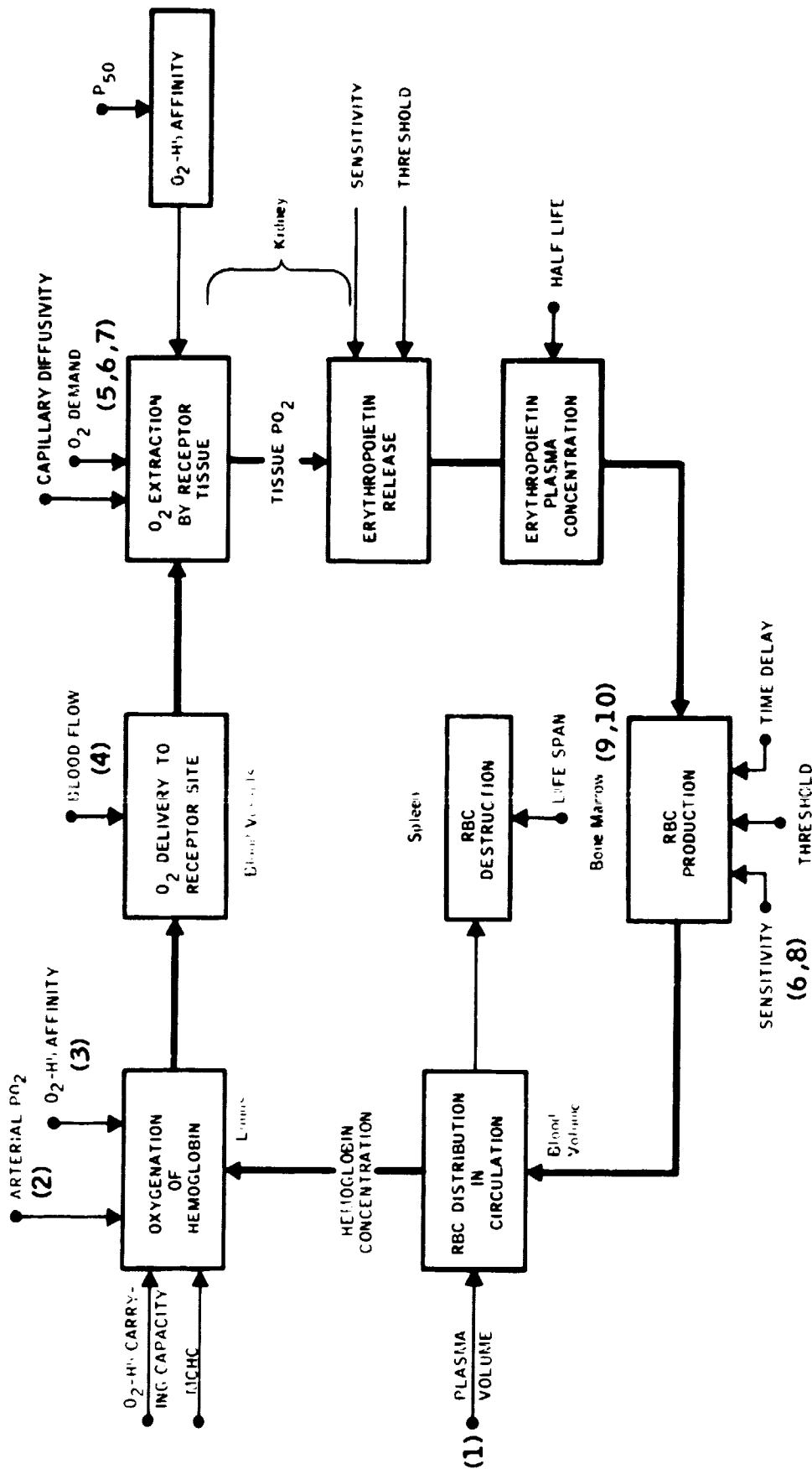


Figure 10: Systems diagram of the model for control of erythropoiesis. Fixed parameters of the model are designated by the bullet-ended arrows. Numbers in parenthesis refer to the parameters that were used to evaluate hypotheses explaining red cell mass loss during space flight (see Fig. 14).

as in Volume II of this report. For convenience of the reader, the major assumptions that were used in formulating the model are summarized in Table 8.

In order to compare the performance of the model to real-world behavior, an understanding of the model's limitations is essential. The model is restricted to control of the erythropoiesis system. It represents a system for controlling levels of circulating red cell mass, but does not have circulatory, respiratory, or biochemical feedback elements which describe the other aspects of tissue oxygenation that are suggested in Figure 8. While user-controlled parameters which represent these functions (i.e., blood flow, arterial oxygenation, oxy-hemoglobin affinity) are included in the model, they are not automatically adjusted in closed-loop autoregulatory fashion.

The anatomical representation of the kidney structure with respect to intrarenal gradients of oxygen tension are not included in the model. This formulation must await further experimental description of the renal oxygen sensing sites. At present, this does not seem to be a serious limitation, either in simulating space flight or related experiments.

The effects of viscosity and blood volume on blood flow and erythropoiesis are not included in the model. The conditions under which these omissions would lead to serious error are not usually encountered in hypogravic simulations. However, since blood flow is an explicit parameter in the model, hypotheses concerning viscosity and volume effects can be, and were, evaluated.

Development of the model and a study of its simulated behavior led to identification of several important features of the control system which are not generally recognized. These properties, derived from a theoretical model, were especially useful in interpreting the experimental results examined in this study, and are summarized as follows.

First, the variable which is under primary control is the renal tissue oxygen tension. Other time-varying quantities, including hemoglobin concentration (or hematocrit) and red cell production rate can be considered feedback variables which are adjusted to return tissue pO_2 toward normal. Erythropoietin acts as a simple messenger conveying information regarding the changing state of renal tissue pO_2 to the bone marrow.

Second, it is assumed that the renal tissue functions as an oxygen sensor which is located at the venous capillaries in a region of constant blood flow and constant oxygen uptake. If other parameters affecting oxygen transport (such as arterial pO_2 and hemoglobin saturation) are non-varying, the sensor

TABLE 8

MAJOR ASSUMPTIONS OF COMPUTER MODEL
OF ERYTHROPOIESIS CONTROL

- o Erythropoiesis is governed by level of renal tissue oxygen tension
- o Decreasing renal oxygen supply in relation to renal oxygen demand results in reduced oxygen tension and increasing rates of erythropoiesis
- o Renal tissue oxygenation is a function of hemoglobin concentration, blood flow, mean corpuscular hemoglobin concentration, oxygen saturation of arterial hemoglobin, diffusivity of oxygen at renal capillaries, and oxygen uptake of renal tissues
- o The hormone, erythropoietin, is released by the kidneys into the blood at a rate inversely related to the renal oxygen tension
- o The rate of red cell production is directly related to the log of the erythropoietin plasma concentration
- o The rate of red cell destruction is based on the lifespan of the cell and is a fixed percentage of the circulating red cell mass
- o Changes in circulating red cell mass are determined by the time integral of the production rate minus the destruction rate

may be considered to function as a hemoglobinometer. In this mode, hemoglobin concentration is the major variable to influence, and ultimately to be controlled by, the oxygen sensor.

Third, the quantity $d(RCM)/dt$ (i.e., the change of red cell mass with respect to time) operates as if it were under integral control so that it always returns to a value of zero at steady-state, regardless of the type of disturbance. This is accomplished by changes in both production and destruction rates.

Fourth, the combined renal-bone marrow controller operates according to the principles of proportional control, at least in its normal operating range. Under this type of control, the tissue oxygen tension, if disturbed away from its control value, will always tend to return to normal, and in most all cases there will be some steady-state error; i.e., 100 percent compensation in the face of a load disturbance is normally not possible. Increasing controller gain (i.e., the sensitivity to tissue pO_2) will decrease the time at which maximum compensation is reached and will decrease the steady-state error at the sacrifice of some dynamic stability in the formation of erythrocytes.

Finally, disappearance of red cells during space flight may be expected to occur under certain conditions at much slower rates than their subsequent replenishment. The maximum rate of red cell mass loss (assuming production is completely inhibited) is limited by the natural attrition rate of red cells which in the human is 1 percent per day or about 20 ml packed red cells per day. In contrast, the maximum rate of red cell production is governed by the bone marrow production capacity, usually said to be about six times normal or about 120 ml packed red cells per day.

One of the first, and potentially the most important, uses to which the model was applied concerned identification of factors which could lead to suppression of circulating red cell mass. In keeping with the emphasis of the previous discussion on tissue oxygenation, and in accord with the model shown in Figure 10, it was obvious that increasing levels of tissue oxygen tension would provide a diminished stimulus for erythropoietin production. The steady-state relationship between tissue pO_2 and red cell production may be derived by reducing the formulation of the model's erythropoietin and erythrocyte controllers (Figure 11a) to the more compact form of Figure 11b. This relationship is shown in more detail in Figure 12, in which only the

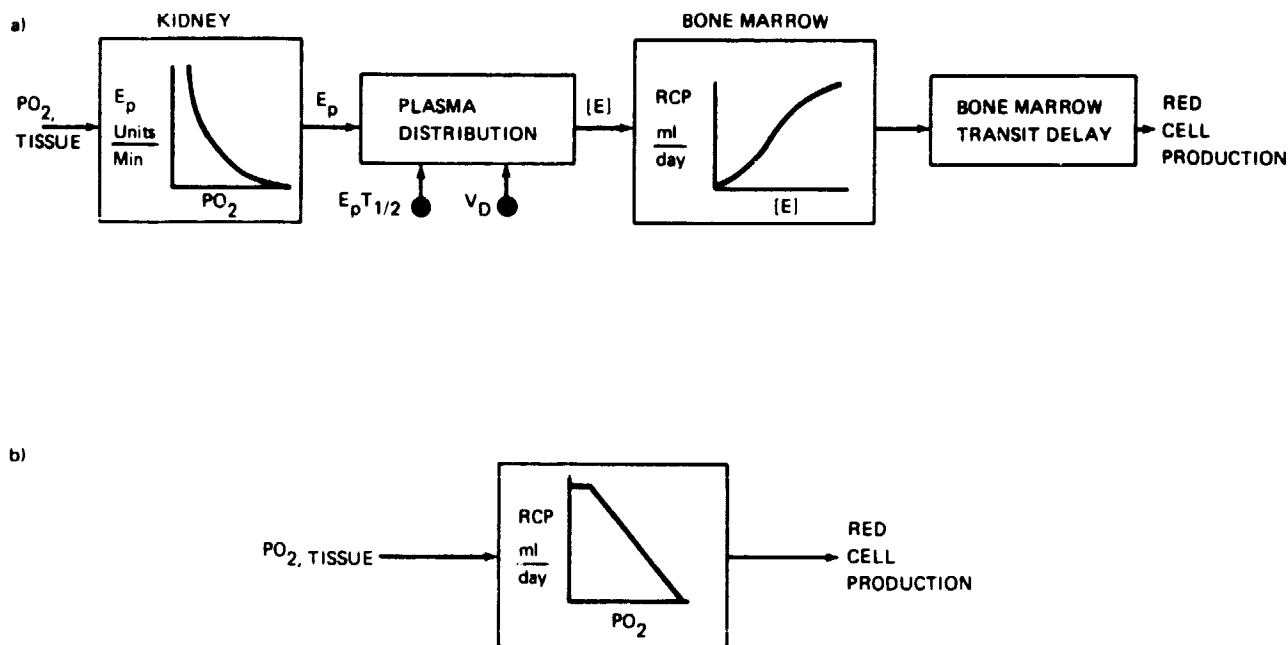


Figure 11: Relationship between tissue oxygenation and red cell production. (a) elements, contained in the computer model, showing the renal-bone marrow controllers which have been simplified in (b) by eliminating the effect of erythropoietin which acts merely as a signal transmitter of tissue oxygen content. A portion of the graph shown in (b) is presented in more detail in Fig. 12. (E_p = rate of erythropoietin release, $E_p T_{1/2}$ = plasma half-life of erythropoietin, $[E]$ = erythropoietin plasma concentration, PO_2 = tissue oxygen tension, V_D = volume of distribution of erythropoietin, RCP = red cell production rate).

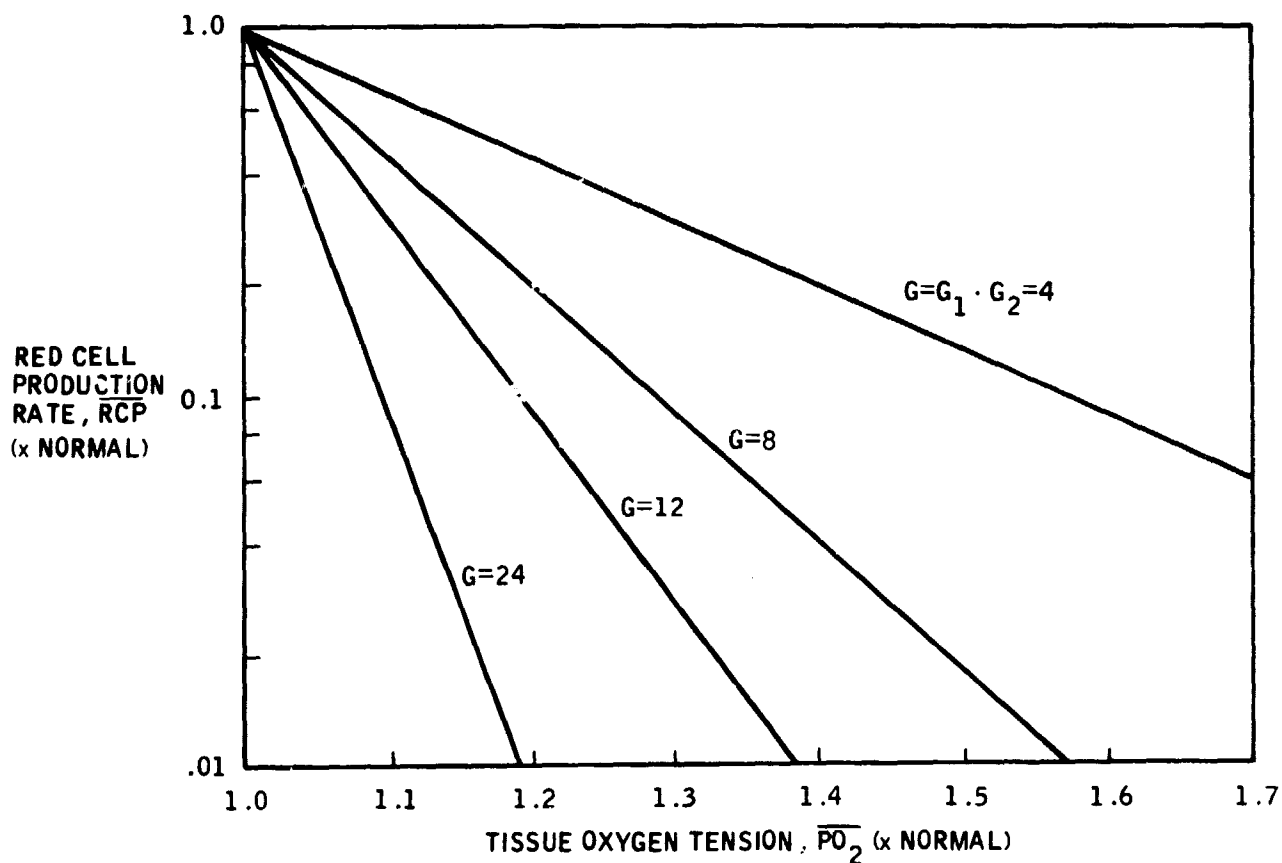


Figure 12: Combined renal-bone marrow controller function curves (semi-logarithmic plot) showing depressed erythropoiesis at high tissue oxygen tensions. Curves for different assumed values of overall controller gain (G) are shown.

hyperoxic range of tissue pO_2 is shown. It is in this region that suppression of red cell production is predicted to occur to a degree dependent not only on tissue pO_2 , but also on the overall controller gain, G . Although neither tissue pO_2 nor controller gain is normally measured directly, these quantities can be determined by parameter estimation techniques. Using the theoretical analysis of Figure 12, it is possible to predict a significant suppression of erythropoiesis due to moderate increases in tissue oxygen tension.

The factors which are capable of altering tissue oxygen may also be assessed for their quantitative and dynamic influence on erythropoiesis using the model. The simulated model behavior resulting from step changes in six normally fixed parameters is shown in the sensitivity analysis of Figure 13. Alterations in the following parameters were evaluated: oxygen uptake of renal tissue, oxy-hemoglobin affinity (expressed as P_{50}), renal blood flow, arterial oxygenation, total plasma volume, and size of the marrow pool (indicated in Figure 13 as bone marrow responsiveness). The values of these quantities were changed prior to simulation in a direction that led to a suppression of erythropoiesis and red cell mass.

The sensitivity analysis provided a basis for determining the relative importance of parameters and for identifying likely causal factors for the decrease in red cell mass observed in space flight. These assessments were considered in the light of known physiological function. For example, while renal oxygen demand demonstrated the most significant effect on tissue pO_2 and erythrocyte production, there was no reason to initially believe that this parameter was altered during exposure to weightlessness. On the other hand, decreases in plasma volume are a well-known occurrence in hypogravity, and the resulting hemoconcentration is capable, at least according to the model's predictions, of producing tissue hyperoxia, suppression of erythropoietin, reduction in erythrocyte production, and gradually diminished red cell mass. In this manner, each of the parameters identified by the systems analysis were evaluated to determine whether a reduced gravity environment could cause a change from its preflight value. The results of manned space-flight experiments were reviewed as well as experimental analogs of long-term weightlessness, such as human bed rest and animal dehydration studies. Factors other than those directly contributing to tissue oxygenation were also considered, such as increased red cell destruction and altered controller sensitivity. Ground-based experiments, such as descent from altitude and red

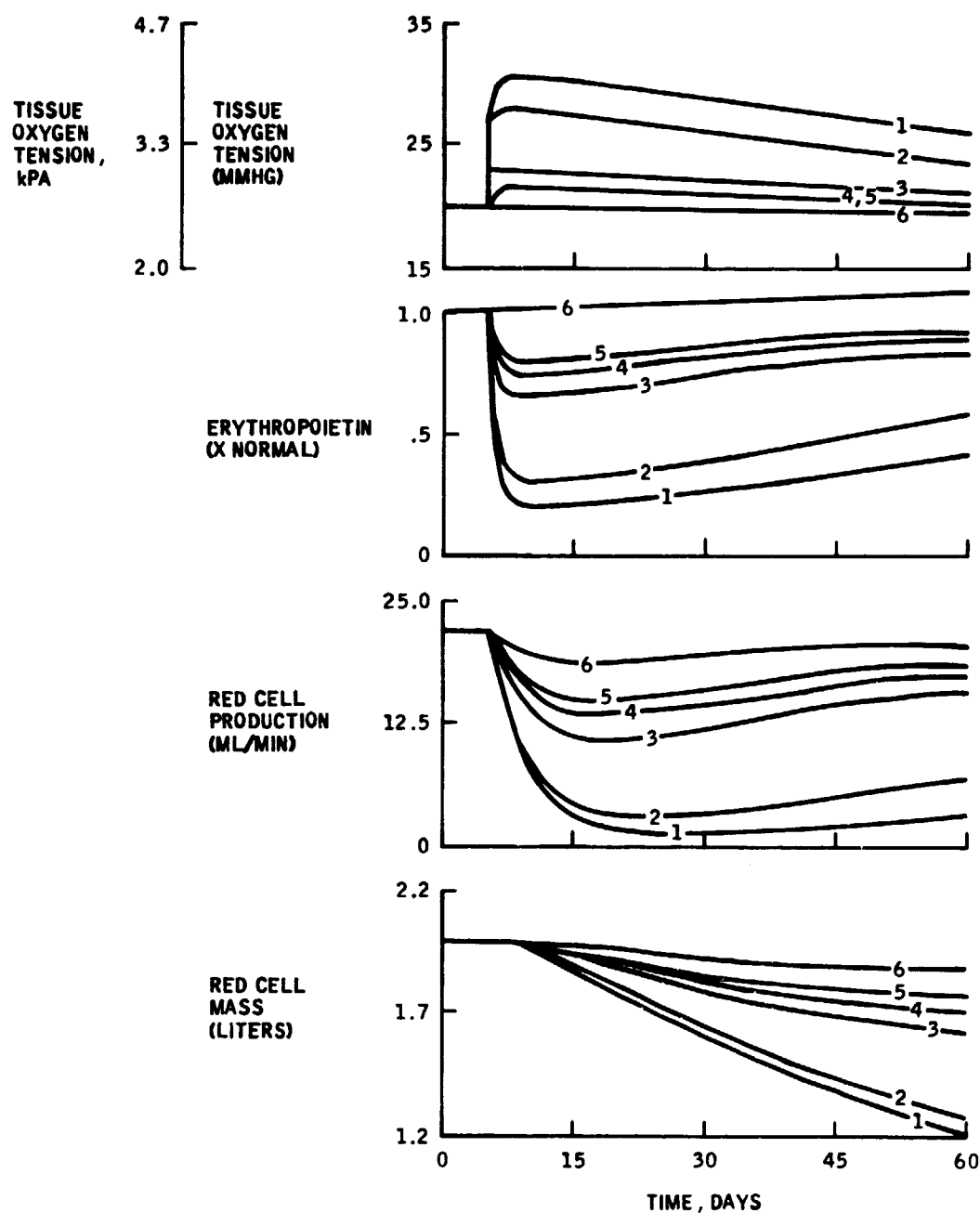


Figure 13: A sensitivity analysis showing the behavior of the computer model resulting from disturbances that lead to suppression of red cell production. The model's response is illustrated for a 20 percent constant load disturbance (that is, step change) of six parameters. Curve 1 = renal oxygen uptake (-), curve 2 = oxy-hemoglobin affinity, P50 (+), curve 3 = blood flow (+), curve 4 = plasma volume (-), curve 5 = arterial oxygen tension (+), curve 6 = bone marrow responsiveness, P1 (-). Algebraic sign refers to direction in change of each parameter from its normal control value.

cell transfusions, were also examined by computer simulation and found to have unexpected relevance to the space-flight situation. This systematic approach (see Figure 1) led to the formulation of a series of hypotheses that could explain zero-g induced red cell loss, not as some pathological event, but in terms of a normal response to disturbances in a feedback control system. These hypotheses and their evaluation using simulation are discussed in the following pages.

DEVELOPMENT OF HYPOTHESES

The most attractive candidate hypotheses for reconciling the experimental findings can be conveniently grouped into three categories: those relating to disturbances in the oxygen-supply demand balance and which are expressed via diminished levels of erythropoietin; those having a direct effect on the bone marrow and thereby by-passing the erythropoietin mechanism; and those which have an influence on removing red cells from the circulation. As illustrated in Figure 14, these hypotheses attempt to explain the disappearance of circulating erythrocytes by either a decrease in production or an increase in destruction.

The mathematical model was particularly well-suited for identifying and evaluating factors relating to oxygen supply. These factors included alteration in Hb concentration, arterial pO_2 , oxy-hemoglobin affinity (i.e., P_{50}), and blood flow. Other factors which may influence oxygen demand (i.e., diminished caloric intake, activity, and diminished lean body mass), the bone marrow (i.e., ineffective erythropoiesis, altered marrow responsiveness, and diet), and red cell destruction (i.e., intravascular hemolysis, hemorrhage, and splenic sequestration) are not explicitly represented in the model. Therefore, inferences regarding these mechanisms were made and were tested in the model by adjustments in the oxygen uptake, bone marrow control function and red cell lifespan parameters. Each hypothesis in Figure 14 is indexed with a number which corresponds to a model parameter (shown in Figure 10) by which the hypothesis could be evaluated.

The following discussion will address each major hypothesis in turn, with particular emphasis on the physiological pathways involved, the evidence which supports the hypothesis, and its plausibility in the context of weightless space flight. Wherever possible, this discussion will be supplemented by computer simulations in an attempt to provide some notion as to the

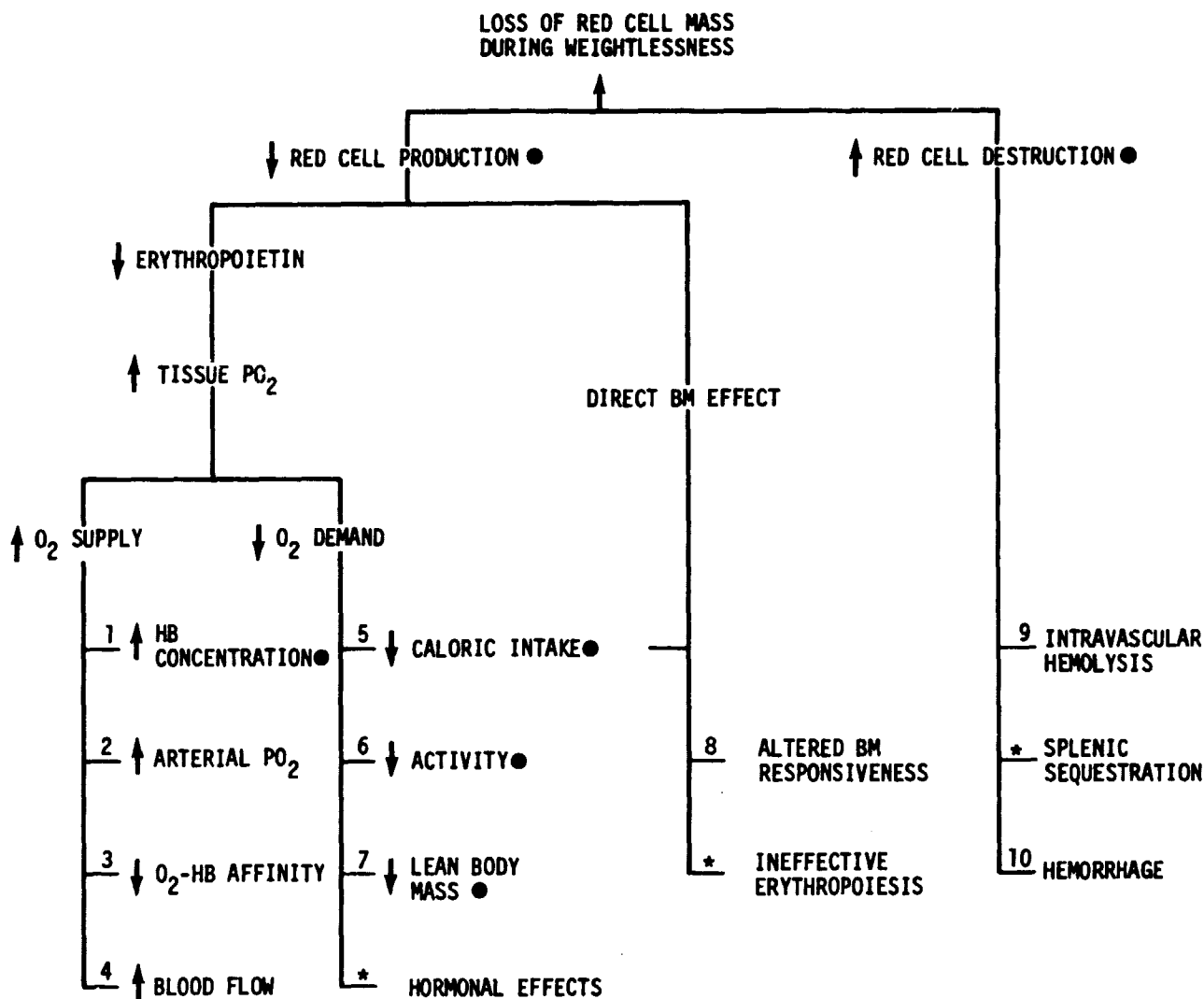


Figure 14: Hypothesis diagram showing the various factors considered in the present study to account for red cell mass loss during weightlessness. The numbers adjacent to each factor indicates the model parameter that was altered (see Fig. 10) in order to evaluate the influence of that factor by simulation techniques. (*) indicates that the designated pathway is not testable in the current model. (●) indicates that evidence is available confirming the primary event shown. (BM = bone marrow, HB = hemoglobin).

quantitative importance of each proposed mechanism. Only simple model disturbances (such as step changes in parameter values) will be used in this discussion. More complex challenges to the model, including multiple, sequential, and time-varying driving functions will be described later when simulations of laboratory experiments are presented.

Hemoconcentration

Of all the factors which can contribute to an increase in oxygen delivery to tissues, there appears to be only one which has been observed consistently in hypogravic maneuvers in association with loss of red cell mass. That factor is hemoconcentration and results from an apparently rapid reduction in plasma volume. Modest elevations in hematocrits up to 15 percent have been observed during space flight, bed rest, and water immersion (Kimzey, 1977; Kollias et al., 1976; McCalley, 1964).

The idea that hemoconcentration was implicated in the hypogravic response was first proposed by Morse (1967) in the interpretation of bed-rest data (see also Lancaster, 1971). Unfortunately, this concept was not related to the space-flight situation until much later (Leonard, 1974; Kimzey, 1977). Other than in bed rest, information relating to the long-term effects of decreased plasma volume on erythropoiesis is sparse. However, there is considerable evidence to suggest that the erythropoietic system is very sensitive to hematocrit changes in either direction. Significant reductions in erythropoietic activity have been observed following the increases in hematocrit induced by red cell infusion in humans (Birkhill et al., 1951) and animals (Gurney and Pan, 1958; Dunn and Lange, 1979), thirst dehydration (Kilbridge et al., 1969; Dunn, 1978) and altitude hypoxia followed by descent to sea level (Buderer and Pace, 1972; Huff et al., 1951). As for decreased hematocrit changes, the enhanced erythropoietic response of many types of acute and chronic anemia are well known (Adamson, 1968; Erslev, 1975). The studies cited were concerned with changes lasting several days to many weeks, and they represent stresses for which the model has been validated. These simulation studies will be summarized later in this section.

Hemoconcentration is not only associated with an increase in viscosity, but also it invariably is produced by events which also alter the blood volume. An increase in hemoglobin level can be experimentally or clinically obtained by reduction in plasma volume (hypovolemic) or transfusions of red

cells (hypovolemic). Both blood volume and viscosity are capable of influencing tissue oxygenation and erythropoiesis independent of hemoconcentration effects, as discussed previously (see Figure 9). It was necessary to account for these viscous and volume effects to explain some short-term hematological observations involving hypovolemic and hypervolemic hemoconcentration (see "Dehydrated Mouse Simulations"). However, the analysis presented in the following pages as well as by others (Kilbridge et al., 1969) suggest that in long-term studies, changes in blood volume or red cell mass by themselves, have much less influence on erythropoiesis than do moderate changes in hematocrit.

According to the regulatory concepts outlined previously, hemoconcentration is capable of reducing bone marrow erythropoietic activity via tissue hyperoxia and suppression of erythropoietin release. This effect was illustrated by model simulations already presented (Figure 13, curve 4) and is further demonstrated by the parametric analysis of Figure 15. In this latter study, the plasma volume parameter was reduced incrementally and simulations were performed at each level for sixty-day periods. As shown in Figure 15, the rapid rise in hematocrit initiated, in turn, the more gradual responses in erythropoietin, erythrocyte production and red cell mass. The falling red cell mass eventually lowered the hematocrit toward control levels, thus diminishing the stimulus for erythropoiesis suppression.

An idealized simulation of space flight or bed rest is illustrated in Figure 16. The two-phase sequence of hypogravity and recovery is characterized, in this case, solely by a period of hemoconcentration, followed by a period of hemodilution. This simulation was driven by an idealized plasma volume behavior (derived from a bed-rest study by Morse, (1967)). Although hemoconcentration causes red cell mass to fall and hemodilution restores red cell mass, the recovery period is not an exact mirror image of the treatment (i.e., bed rest) phase. Many factors contribute to this non-symmetrical effect, including non-linear controller functions, time delays in renal and bone marrow elements, and the fact that the hemodilution event is initiated from an erythro-suppressive state in contrast to the normal state preceding hemoconcentration.

If hemoconcentration is a major factor to consider in explaining losses in red cell mass, then it is also important to examine the degree and dynamic behavior of the hemoconcentrating event. In bed rest, hemoglobin levels tend

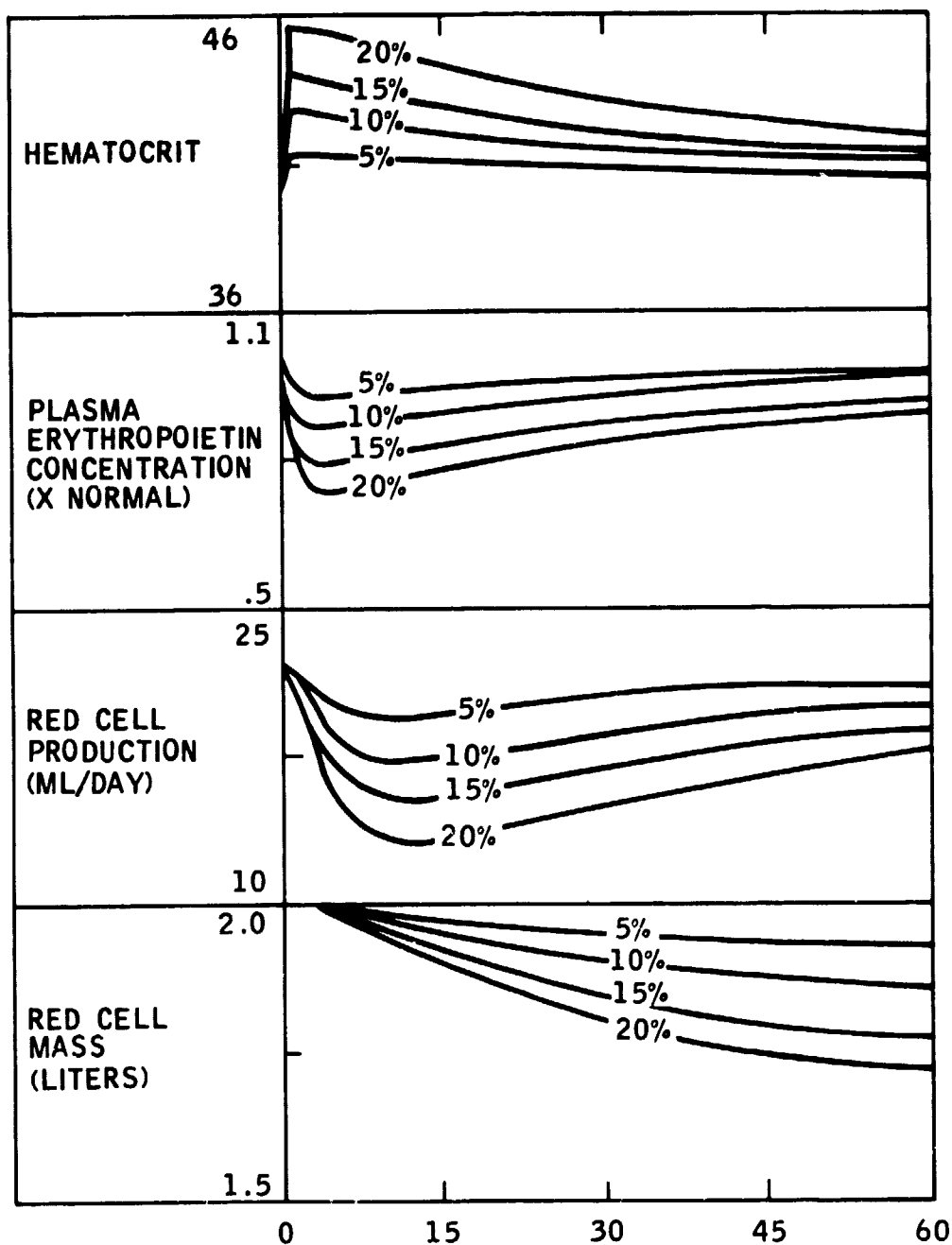


Figure 15: The simulated effect of hemoconcentration on erythropoiesis regulation illustrating the sensitivity of the model to changes in plasma volume. The plasma volume parameter was diminished at zero time by the percentage indicated and held at that level for the entire period shown. Gain of the feedback controller was set to $G=6$ for this and all other simulations in this section unless otherwise indicated. Increasing the gain value would cause a similar but more intense response leading to even larger losses of red cell mass. The effect of gain on erythropoiesis is indicated in Figure 12.

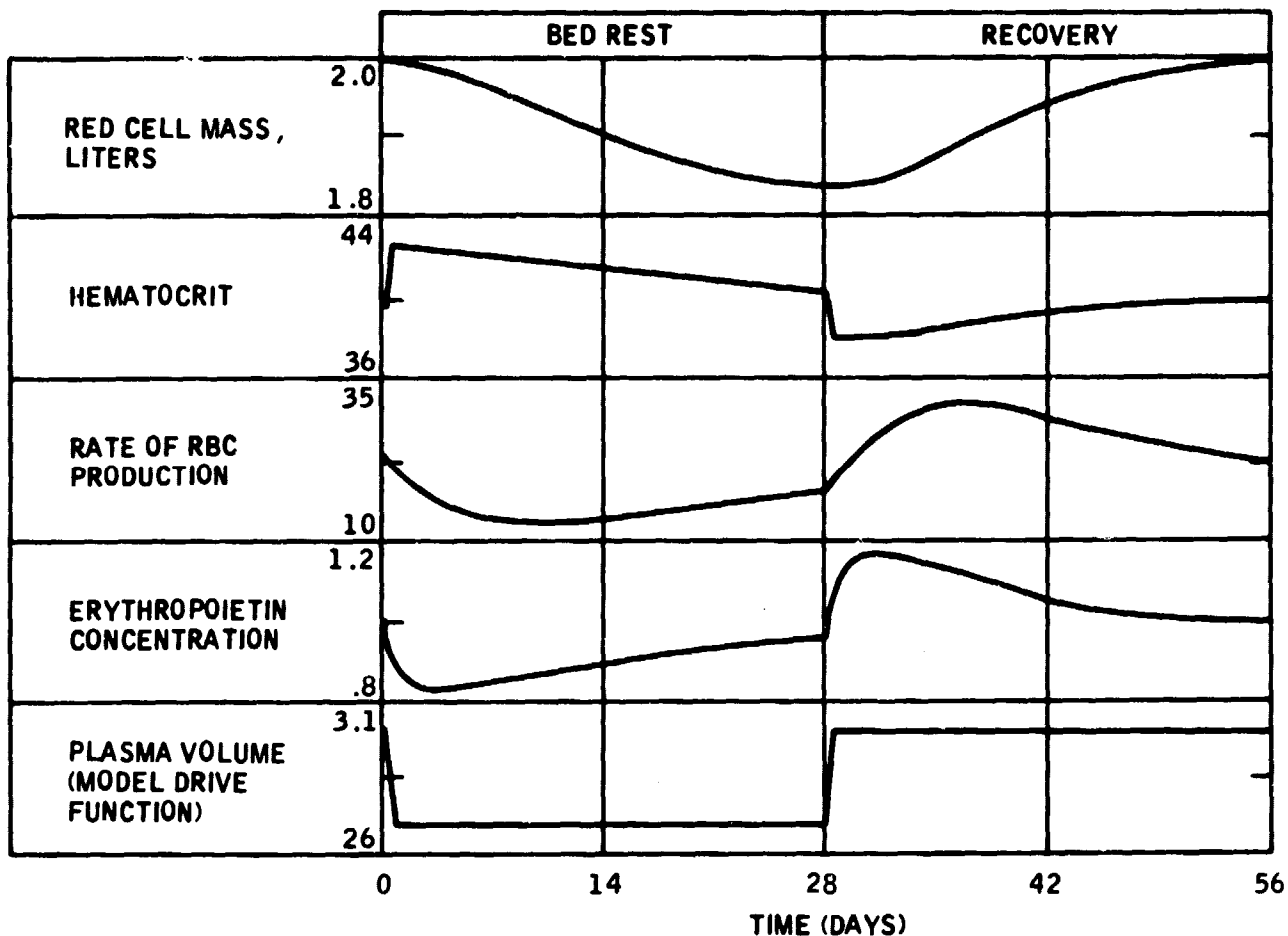


Figure 16: Simulation of a hypothetical bed-rest study in which plasma volume is reduced 300 ml (-10 percent) and then returned to normal after 4 weeks. Changes in erythropoietin, red cell production, and red cell mass occur as a result of alterations of hematocrit.

to rise slowly and then stabilize, while in space flight the rise is relatively more rapid and extreme in magnitude, and a subsequent prolonged downward trend toward control is observed (see Figure 4 to 6). Comparable changes in plasma volume losses (10-20 percent) can occur in both space flight and bed rest, but the reduction in volume proceeds more rapidly in space flight. This difference in rate of plasma loss will, of course, be reflected in the dynamic behavior of hemoglobin levels, so that the hemoconcentrating ability of space flight is initially greater than that for bed rest. The long-term behavior of hemoglobin levels depends entirely on the relative rates at which plasma volume and red cell mass disappear. As will be shown later, the computer model proved to be an ideal tool to explain these complex dynamics and to examine the erythrokinetic differences between bed rest and space flight.

Arterial Oxygen Tension

While the atmosphere of the Skylab workshop did not contain oxygen in high enough concentrations to produce toxic effects on red cells, it did exert a higher than normal oxygen partial pressure (189 mmHg compared to 160 mmHg). If this resulted in even modest increases in blood pO_2 , the possibility exists that red cell production was partially suppressed as a result of decreased levels of erythropoietin secondary to tissue hyperoxia (Jaskunas et al., 1973; Larkin et al., 1972). A second factor present that could contribute toward a higher than normal oxygen partial pressure in the blood was an observed resting ventilatory rate (minute volume) that was about 20 percent higher inflight compared to preflight (Michel et al., 1977).

While the inflight pO_2 of Skylab's atmosphere was approximately 30 mmHg higher than preflight levels the effective increase in blood pO_2 would be expected to be much less because of a modulating effect in lung oxygenation. In the absence of direct blood measurements, a formulation for predicting blood pO_2 from ambient oxygen concentrations was employed (Malkin, 1975). The results of applying this equation to the three Skylab crews are shown in Table 9. On the average, there was an estimated increase of only 3-4 mmHg in blood pO_2 during the flight phase in comparison to the preflight phase. The crew of the 28-day mission showed the highest estimated arterial pO_2 . The fact that this crew also exhibited the largest losses in red cell mass may be more than coincidental.

TABLE 9
ESTIMATES OF PARTIAL PRESSURE OF OXYGEN IN ARTERIAL BLOOD
OF THE SKYLAB CREW*

Quantity	Preflight	I N F L I G H T			
		28-Day Mission	59-Day Mission	84-Day Mission	Skylab Avg.
B**	760	252	263	259	258
$P_{I}O_2^{**}$	158	194	185	189	189
$P_{I}CO_2^{**}$	0	3.7	5.0	5.1	4.6
$P_{A}CO_2^{**}$	40	43.7	45.0	45.1	44.6
RQ **	0.87	0.91	0.90	0.88	0.90
P_aO_2	109.5	117.5	110.4	114.1	113.1
P_aO_2	0	8.0	0.9	4.6	3.5

* P_aO_2 =

where B = total ambient pressure, mmHg

PH_2O = partial pressure of water vapor in lungs (at body temperature of 37°C, PH_2O = 47 mmHg)

$P_I O_2$ = ambient partial pressure of oxygen, mmHg

$P_A CO_2$ = partial pressure of CO_2 in alveolar air, mmHg (assumed that $P_A CO_2 = 40 + P_I CO_2$)

RQ = respiratory quotient

$P_a O_2$ = partial pressure of oxygen in arterial blood, mmHg

$P_a O_2$ = partial pressure of oxygen in arterial blood, mmHg

$P_a O_2$ = difference in $P_a O_2$ between inflight and preflight

** measured directly

Even small changes in arterial blood oxygenation can accumulate over time and eventually lead to significant changes in total circulating red cell mass, as suggested by the simulations of Figure 17. If atmospheric hyperoxia is combined with bed rest or space flight, a larger decrement in red cell mass may, therefore, be expected than for bed rest or space flight alone. This has not been confirmed experimentally, but the reverse case - altitude hypoxia during bed rest - demonstrated that hypoxia can abolish the erythro-suppressive effects of hemoconcentration (Stevens et al., 1966).

The evidence reviewed here is indirect and suggestive, so that a strong conclusion regarding the effect of enhanced arterial pO_2 is not warranted. However, it may have been a contributory factor, especially in the largest red cell mass losses observed on the first Skylab mission.

Oxy-Hemoglobin Affinity

Displacements of the oxygen-hemoglobin equilibrium curve (OEC) to the right or left (conventionally characterized by the value of P_{50}) reflect decreasing and increasing affinity, respectively, between oxygen and hemoglobin. Any change in position of the OEC profoundly affects the amount of oxygen available at normal tissue oxygen tensions, whereas oxygen uptake by hemoglobin is little affected (Finch and Lenfant, 1972). The capability to alter O_2 -Hb affinity and oxygen unloading at the tissue level in response to environmental changes and ultimately influence erythropoiesis regulation has only recently been appreciated (Astrup et al., 1968; Beutler, 1969; Parer, 1970).

Qualitatively, a decrease in oxygen-hemoglobin affinity can be considered comparable to increases in blood flow, hemoglobin concentration, or arterial pO_2 in promoting the supply of oxygen to tissues. Changes leading to a shift to the right (increasing values of P_{50}) have been observed in subjects exposed to hypoxic conditions at high altitude, exercising individuals, and patients with anemic and cardiac impairment (Brewer and Eaton, 1971; Lenfant et al., 1971; Kennedy and Valtes, 1954; Woodson et al., 1970). The reverse situation, leftward shifts in the equilibrium curve in response to hyperoxia, may be expected on theoretical grounds, but supportive evidence is scant. The outward appearance of a feedback control circuit is, therefore, present,

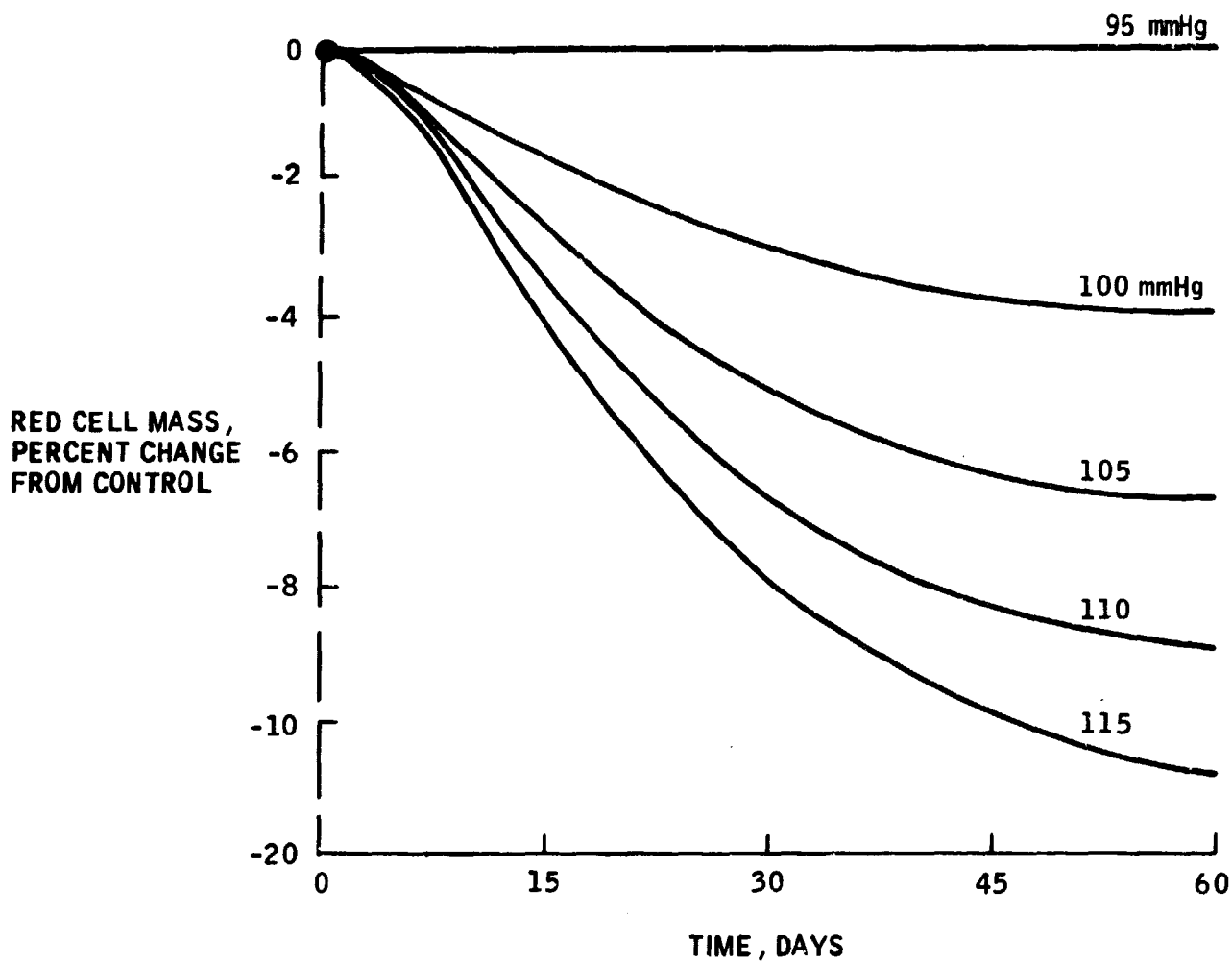


Figure 17: The simulated effect of increasing arterial blood oxygen tension (P_{O_2}) on reducing red cell mass. At zero time the value of P_{O_2} was increased from its normal value of 95 mmHg to the value shown in the graph and it was held at that level for the entire period shown. The behavior of other model variables during this simulation is indicated in Fig. 13 (curve 5).

whereby tissue hypoxia increases blood P_{50} , which facilitates oxygen unloading from blood hemoglobin, thus tending to correct the hypoxic disturbance.

The influence of shifts in the OEC on erythrocyte production has not been well studied, but this relationship is suggested from reports relating P_{50} levels to erythropoietin production (Parer, 1970; Adamson et al., 1969; Hillman and Finch, 1974). A pathologically or experimentally induced shift of the equilibrium curve could conceivably result in anemic or polycythemic conditions (Beutler, 1969). Simulations using the computer model support the view that small shifts in P_{50} cause dramatic short-term changes in tissue oxygenation and long-term changes in red cell mass (e.g., Figure 13; curve 2).

It is well known that the position of the OEC is governed by such blood factors as pH, CO_2 , and temperature. An additional factor that has more recently been implicated in oxygen-hemoglobin affinity alterations are intracellular phosphate compounds, particularly 2,3-disphosphoglycerate (2,3-DPG) (Duc and Engel, 1969). The dissociation of oxygen appears to be facilitated by 2,3-DPG because it preferentially and reversibly binds deoxyhemoglobin. The shifts in P_{50} , discussed here in relation to hypoxic situations are believed to be a result of increasing levels of 2,3-DPG (Brewer, 1974; Bunn and Jandl, 1970).

There is no conclusive evidence that hemoglobin changes of this nature can account for the loss of red cell mass during space flight or bed rest. Values of P_{50} during hypogravity maneuvers have not been reported. Skylab measurements suggest that an increase in inflight 2,3-DPG may have occurred (Mengel, 1974; Kimzey, 1979). Also, plasma hyperphosphotemia was observed during space flight (Johnson et al., 1977) and the possibility exists that blood pCO_2 levels were increased because ambient levels of carbon dioxide were many times normal concentrations in the artificial gaseous atmosphere (see Table 9). Both of these may contribute toward a decrease in oxygen-hemoglobin affinity and a tendency for unloading additional oxygen at the tissue level. On the other hand, if the feedback concept outlined above is valid, the modest degree of predicted tissue hyperoxia (caused by hemoconcentration) would have contributed toward an increased oxy-hemoglobin affinity. These opposing considerations prevent one from reaching a conclusive prediction regarding alterations of inflight oxy-hemoglobin affinity. However, during the postflight recovery phase, it seems more likely that the hemodilutional anemia

would result in a feedback increase in P_{50} . This latter hypothesis was tested by computer simulation and appears to be a fruitful area for experimental investigation.

Renal Blood Flow

Renal blood flow could markedly affect the amount of oxygen delivered to the special oxygen sensing sites. If tissue oxygenation of renal tissue is similar to that of other tissues, a reduction of renal flow would decrease tissue oxygen tension (Granger et al., 1975). Moreover, since erythropoietin release is supposedly a sensitive indicator of renal tissue oxygenation, increasing amounts of erythropoietin should be observed in tissue made hypoxic by reductions in renal arterial flow. (This is a prediction of the computer model as inferred by Figure 13.) Some experimental studies using renal artery occlusion support this hypothesis (Fisher et al., 1965; Takaku et al., 1962) while others do not (Krantz and Jacobson, 1970; Gordon and Zanjani, 1970; Adamson and Finch, 1975).

Two factors in particular may help explain a relative insensitivity of the erythropoietin producing apparatus to renal blood flow. First, in contrast to all other body organs and tissues, the oxygen demand or consumption in the kidney is more or less proportional to blood flow rather than remaining relatively constant at rest (Pitts, 1968). Thus, although moderate reductions in renal blood flow do affect oxygen delivery, they may not appear to alter tissue pO_2 and erythropoietin production, presumably because renal oxygen consumption is also decreased. Secondly, renal blood flow is known to be under a high degree of autoregulatory control (Selkurt, 1963). Both of these considerations suggest that the balance between tissue oxygen supply and demand is not markedly influenced by moderate blood flow changes in the kidney. Whether the renal oxygen detector sites exhibit these same characteristics that may exist for the kidney as a whole is not known.

In order to explain the space-flight decrease in red cell production on the basis of blood flow, an increase in renal blood flow must be postulated. There is some evidence, based on postural change studies, which suggests an increase in renal blood flow during the acute stress phase of weightlessness (Pitts, 1968; Piemme, 1968). The headward shift of fluid is a volume-expanding event with respect to the upper body circulation and may enhance renal flow, at least temporarily. However, there is some indirect evidence

that does not support an increased renal blood flow, or at least confuses the issue. First, an increased renal blood flow is not a consistent finding in acute water immersion studies (Epstein, 1978), and second, longer term bed-rest studies show equivocal results with respect to changes in renal blood flow (Fuller et al., 1976; Melada et al., 1975), although none of these studies measured erythropoietin or the direct effect on erythropoiesis. Third, exercise is known to decrease renal blood flow (Selkurt, 1963) and daily exercise of varying levels of intensity was used as a deconditioning countermeasure on Skylab flights. Fourth, the hemoconcentration accompanying space flight may be responsible for altering intrarenal blood flow (Migdal et al., 1975) and, therefore, may affect the renal oxygen sensors in a yet unknown manner.

In view of the inconclusive evidence relating blood flow to erythropoiesis and the lack of data describing renal blood flow change during space flight, it would seem prudent to await new experimental evidence before invoking this mechanism as a long-term causative factor in the reduction of red cell production.

Altered Oxygen Demand

A decrease in red cell mass could conceivably result from decreased oxygen consumption of the renal tissue because erythropoietin is supposedly responsive to the oxygen supply-demand relationship in that tissue. The major portion of renal oxygen demand is directed toward active transport processes related to eliminating wastes and conserving nutrients (Pitts, 1968). Any event that decreases waste product or nutrient supply to the kidney may be expected to reduce renal oxygen uptake. Such events might include the restrictions in dietary intake and reduced metabolic activity in bed-rest subjects and in astronauts in confining spacecraft. Although a reduced level of activity was not believed to have been present during Skylab, dietary levels were inadequate throughout the 28-day mission and possibly the 59-day mission, and particularly for the first week of flight on most other missions, since some of the crewmen were space sick (Thornton, 1978). On the other hand, loss of cellular material resulting from musculoskeletal atrophy, a common occurrence in both bed rest and space flight, might lead to greater demand for renal oxygen uptake in ridding the body of this waste material. Therefore, the net effect on renal oxygen consumption is difficult to predict.

It is also reasonable to examine possible changes in whole-body oxygen demand, since this parameter is more easily measured and has been linked to changes in erythropoietic activity. For example, the decreases in metabolic rate seen with hypophysectomy, starvation, and hypothyroidism reduced the need for oxygen in relation to an unaltered oxygen supply and were followed by decreased erythropoiesis until a new steady-state was established (Jaobson and Krantz, 1970). Decreases in basal metabolism of about 7 percent have been noted in bed-rest subjects, and even larger decrements would be expected in total energy metabolism (Dietrick et al., 1948). Weightless space flight was thought at one time to promote effortless activity in performing tasks with an accompanying reduction in metabolic demand (Rambaut et al., 1973). Consequently, recommended dietary intake was usually reduced below preflight levels. However, this concept has not been borne out and it is now believed that in a high activity mission like Skylab, total metabolic expenditure is similar to that on the ground (Rambaut et al., 1977). The tentative conclusion one draws is that total oxygen consumption is reduced during bed rest and during flights in small space capsules, but there is probably little change for subjects in larger space-flight environments.

Hypotheses regarding altered oxygen demand on space flight erythropoiesis activity were only evaluated to a limited extent in the present study because there is scant quantitative information available for statistical analysis, and there is no information available regarding renal oxygen consumption. Likewise, there is no information presently available that suggests a mechanism by which whole-body oxygen demand can influence erythropoiesis by pathways not related to renal oxygen demand. However, it was desirable to consider changes in oxygen demand in the particular instance of evaluating the wide variation in exercise levels recorded by the Skylab crew (see next page).

Reduced Lean Body Mass

Hypogravity, whether induced by bed rest or by space flight, often results in a decreased lean body mass, even in the presence of periodic exercise (Greenleaf et al., 1977). Among a normal population, there is an excellent correlation between lean body mass and red cell mass (Muldowney and Healy, 1967). It is reasonable to speculate that this correlation may be based on the size of the oxygen-supplying erythron conforming to the size of the metabolically active tissue mass of the body. If this is true, a decrease in

lean body mass may be a causal factor in a reduction in red cell mass. This influence was statistically evaluated in the Skylab crewmen and will be discussed in a later section. One might also conjecture that it is not the size of the lean body mass that is important, but rather the total amount of oxygen consumption by this tissue. Since the limited data available suggests that, total body metabolism is not different in space than on Earth, an alternative hypothesis is that proportionately more work was being demanded of a diminishing muscle mass (Rambaut et al., 1977) and hence, no change in erythron mass would be expected as a result of a reduction in lean body mass.

Effect of Physical Activity

Exercise conditioning is known to have major long- and short-term effects on the oxygen transport systems (Scheuer and Tipton, 1977). Therefore, it is certainly conceivable that exercise, or the lack of it, would affect the erythropoietic process and modify the red cell mass response to both bed rest and space flight. However, the effects of exercise on erythropoietic activity and circulating red cell mass are complex and poorly understood.

No clear picture emerges regarding an exercise effect from a review of space-flight and bed-rest studies. Simple bed rest, characterized by a complete absence of physical activity, is associated with a loss of red cell mass (see Figure 6). When intermittent daily supine exercise was added to a bed rest protocol, the loss of red cells became more severe, at least in one study (Miller et al., 1965). Exercise associated with space flight has been characterized by varying degrees of metabolic activity ranging from partial immobility during the earliest suborbital flights to the shirt-sleeve 90-minute daily workouts on treadmill and bicycle during the 84-day Skylab mission. In Skylab, (and in seeming contrast to the bed-rest study cited above) increasing levels of exercise were correlated with smaller decreases of red cell mass. However, this result is not conclusive because of the lack of suitable controls.

The possibility exists that exercise may have an effect, not only during bed rest or space flight, but during the recovery period as well. During recovery from bed rest and during the first (28-day) Skylab mission, repletion of red cell mass was observed to be delayed for up to two weeks. A delay was not observed after the 59-day and 84-day missions. It has been suggested that these observations may be attributed to the increase in physical activity

following bed rest (Miller et al., 1965; Johnson and Driscoll, 1977) in contrast to a reduction in activity following the 59-day and 84-day Skylab missions.

While most investigators find an increase in blood volume during simple exercise conditioning, there is as yet no clear agreement whether this is accompanied by an increase or decrease in red cell mass (Sjostrand, 1962; Rocker et al., 1976, 1983; Holmgren et al., 1960; Bruce et al., 1975; Scheuer and Tipton, 1977). These inconclusive results pertain to both comparisons between athletic versus non-athletic populations and longitudinal training program studies in the same individual. Most often, an increase in plasma volume (at rest) is observed with exercise training associated with a concomitant decrease in hematocrit. While firm conclusions regarding a consistent effect cannot be drawn from these data, the change in blood volume, including increases in red cell mass, that have been reported, are, on occasion, significantly large.

Physiologically, it appears that exercise may have two opposing effects on the circulating red cell mass that can account for the varied results. On the one hand, sustained physical training regimens may be associated with an increase in blood destruction rates manifested by an observed drop in red cell mass and hematocrit. This phenomenon, first observed in dogs (Broun, 1923) was recently termed "sports anemia" and has been attributed to mechanical hemolysis resulting from heavy muscular work (Radomski et al., 1980). It has been observed in both fit and unfit populations, although the results seem to be exaggerated after prolonged confinement or sedentary activity. On the other hand, exercise may act as a bone marrow stimulant and increase the production of red cells. This phenomenon has not been well described, but possibly is a result of the stimulus of blood loss arising from the hemolysis effect (Broun, 1923). Also, it is plausible to consider the increased need for oxygen as a causative factor.

Tests with the erythropoiesis model have shown that increased destruction of cells will reduce the hematocrit, and this slight anemic condition will, in turn, increase red cell production. The net result, however, is a slightly reduced hematocrit (see Figure 18); that is, over-compensation and net increases in red cell mass do not occur in the model system, as implied by Broun (1923). The simulation analysis confirmed the analysis of Hodgson (1970), who showed on theoretical grounds that large increases in destruction

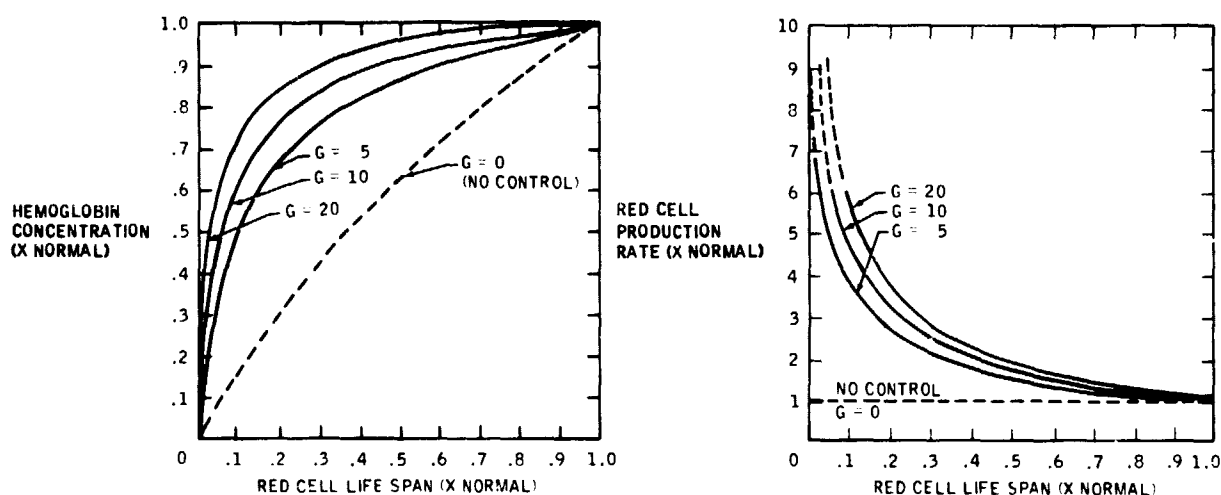


Figure 18: Simulated steady-state response to shortened red blood cell life-span (hemolytic anemia). Predicted hemoglobin levels and red cell production rates are shown as a function of red cell lifespan and controller gain (G). Dashed line ($G = 0$) indicates no feedback control. Results suggest that hemoglobin levels are always below control, but they can be maintained near normal for a large range of reduced lifespan provided that controller gain is high.

rates will result in relatively small decreases in red cell mass because of an effective and sensitive controller of bone marrow production.

If exercise, per se, can influence both destruction and production rates of red cells, then any resulting changes in red cell mass would be dependent on the delicate balance between these two rates. Any imbalance would have an accumulative effect during prolonged training periods. Mechanical destruction of cells would likely be affected by the type of exercise, whether it be jogging, skiing, or swimming. Similarly, if production is based on long-term oxygen demand, the intensity and duration of exercise would be crucial factors affecting steady-state rates of erythropoiesis. These considerations could, in part, explain why different investigators obtain different results. Unfortunately, the most precise methods available to measure rates of red cell production and destruction have yet to be applied to exercise studies.

The effect of complete cessation of exercise in previously athletic individuals, as exemplified in some bed-rest studies and pre-Skylab space flights, may lead to effects opposite to those of exercise training. A decreased metabolic rate, a decrease in lean body mass (see the earlier sections entitled "Altered Oxygen Demand" and "Reduced Lean Body Mass") and minimal mechanical stress could increase red cell lifespan (by reducing the rate of hemolysis in previously athletic subjects), and increase the tendency for lower red cell production rates. Without precise determinations of these opposing factors which influence red cell mass, it would be difficult to predict the final effect.

Any theory that exercise, or the lack of it, influences hematological behavior during hypogravic maneuvers must be consistent with the following facts: bed rest is characterized by a cessation of exercise and loss of red cell mass; the Skylab missions were characterized by a loss of red cell mass that was less severe on each subsequent mission in association with increasing amounts of exercise; the degree of exercise on the shortest Skylab flight was probably less than was performed preflight, and this crew lost the largest amount of red cells compared to any subsequent crew and also exhibited a possible shortening of red cell lifespan at recovery; and, recovery from bed rest (ambulation) and from the 28-day Skylab mission was characterized by increased activity and a delayed recovery of red cell mass.

The following hypotheses are proposed to account for these observations:

- a) some factor other than exercise, possibly hemoconcentration, is responsible for the primary fall in red cell mass during hypogravity maneuvers, but exercise could modify this response;
- b) oxygen demand, as reflected by the degree of activity performed over a long time span during bed rest or space flight, ultimately has a controlling influence on promoting red cell production by an unknown mechanism;
- c) the type and intensity of exercise performed during weightlessness does not lend itself to significant levels of red cell destruction; and
- d) a lack of exercise in certain hypogravic studies may conserve fragile red cells (i.e., increased lifespan) which are more vulnerable to destruction during the early ambulation period (i.e., decreased lifespan) (Johnson and Driscoll, 1977).

These hypotheses are aimed primarily at explaining differences between studies in which exercise levels are varied. Accordingly, the increasing degree of exercise on each longer Skylab mission may have limited the severity of the red cell loss. Also, assuming that a period of high activity precedes and follows a minimal activity bed-rest phase, or a high activity space-flight phase, the exercise effect, as such, could cause red cell loss to be more severe after bed rest than after space flight. Finally, the delay in replenishment of red cells during the recovery period of both bed rest and low activity space flights is in accord with hypothesis (d).

There are some grounds for questioning this set of hypotheses. The postulated effect of long-term oxygen demand on red cell production is not known, but cannot be ruled out, based on available evidence. Such an effect may exist under conditions of reduced red cell mass (that is, weightlessness), but not when red cell mass is normal (as in terrestrial studies of exercise conditioning). Also, evidence for significant destruction of red cells during or following bed rest or space flight is not strong (except under conditions of pure atmospheric oxygen). However, only limited data are available and quantitative techniques for testing these hypotheses suffer from lack of precision.

This discussion of potential exercise effects is presented, in spite of its speculative nature, because it is one of three factors that have been identified which might explain the differences between the red cell losses on each of three Skylab missions. The other two factors are diet and mission duration. These will be considered next.

Effect of Diet

It has been established that animals deprived of food, protein, or water exhibit a significant suppression of red cell production (Reissman, 1964; Anagnostou et al., 1977; Fried et al., 1957; Naets and Wittek, 1974; Giglio et al., 1979). The implication of this finding as a causal factor of the "anemia" of space flight was recently suggested on the basis of hematological findings in dehydrated mice (Dunn, 1978b; Dunn and Lange, 1979a). Restriction of food and water intake was frequently noted on the Skylab mission during the first week of flight (Leonard, 1980). Also, on at least one Skylab flight and on numerous pre-Skylab missions, the diet was considered significantly inadequate (Rambaut et al., 1973; Thornton, 1978). These factors contributed toward a negative energy balance and negative water balance in space flight (Rambaut et al., 1977; Leonard, 1977b), both of which are characteristic findings of the original animal studies.

The actual mechanisms whereby food restriction or dehydration in animals reduce red cell production is not entirely clear. A resolution of the processes involved is complicated by the fact that starved mice or rats refuse water and dehydrated animals refuse food. There appear to be at least two components to the erythroid suppression which follows either food or water restriction: a component related to dehydration and the resulting increase in hematocrit, and an energy-balance factor related to the reduced food consumption (see Figure 19).

With regard to the first component, water deprivation induces a state of relative polycythemia which, by increasing oxygen capacity per unit of blood, could lead to tissue hyperoxia and suppression of erythropoietin (Dunn and Lange, 1979; Giglio et al., 1979). This is similar to the pathway previously described by which polycythemia, induced by red cell infusion or bed rest, reduces erythropoietic activity (Kilbridge et al., 1969; Leonard, 1976).

The energy balance component of red cell loss is more complex and not as well understood. Deprivation of water and food was shown to result in a

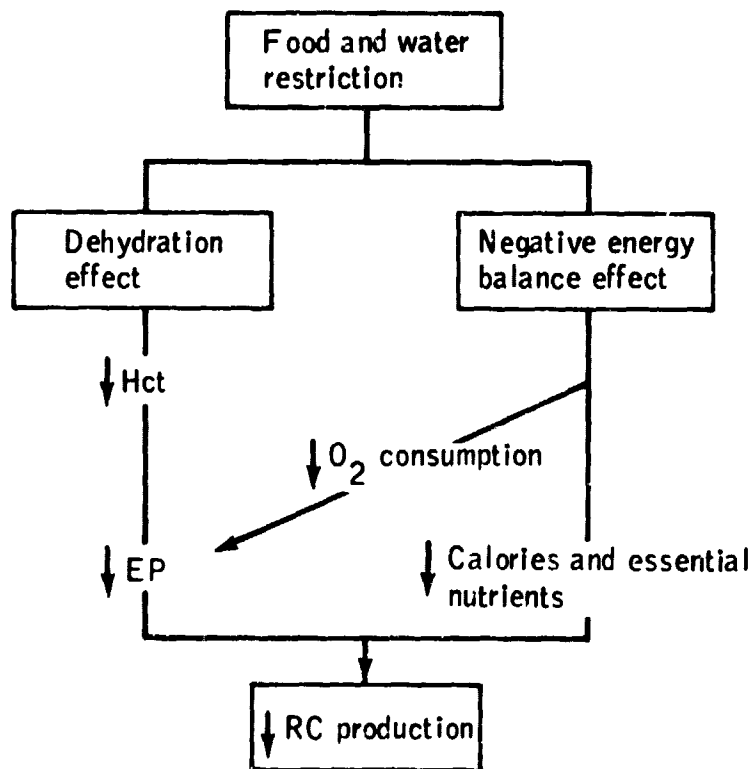


Figure 19: Hypotheses that can account for reduced erythropoiesis following food and water restriction. These pathways are based on animal studies. (Hct = hematocrit; EP = erythropoietin).

depression of oxygen uptake, reduced radio-iron uptake by red cells, decreased erythropoietin formation in hypoxia and reduced erythroid responsiveness to exogenous erythropoietin (Dunn, 1980; Giglio et al., 1979). In terms of the mathematical model of erythropoiesis, these findings can be interpreted to mean that food restriction leads to decreased red cell production by at least three separate pathways (see Figure 19):

- a) a reduction in oxygen demand, which leads to tissue hyperoxia if oxygen supply is constant;
- b) a direct effect on the erythropoietin producing tissues, which reduces their sensitivity to changes in oxygen supply; and
- c) a direct effect on the erythrocyte-producing tissues, which reduces their sensitivity to changes in erythropoietin.

The latter two direct effects on the erythropoietic controllers may be a result of deprivation of critical proteins (Anagnostou et al., 1978). The possibility that a disturbance of food intake can affect the erythropoietic regulatory process at several distinct entry points as shown in Figure 19 makes the negative energy balance mechanism a potentially powerful one. In an effort to distinguish between these different effects, it has been concluded that both the hematocrit and the negative energy balance effects are each significantly important, at least in short-term studies (Dunn, 1980).

It is not clear whether the food deprivation mechanism is operative in humans as it is in animals, and if it is, whether the magnitudes of dietary intake restriction observed in astronauts can produce a similar erythroid-suppression behavior. Also, it is not likely that a dietary effect can explain the entire loss of red cell mass in the Skylab astronauts because a significant loss was noted in the one crew (84-day flight) that was known to have an entirely adequate diet. However, this pathway may explain differences in red cell loss between the different crews inasmuch as the astronauts having the largest negative energy balance also showed the largest decrease in red cell mass.

Altered Controller Function

The shape and position of the renal and bone marrow tissue function curves (see Figure 11) have been found to be crucial elements in the control of erythropoiesis. Each curve (which is essentially a dose-response curve describing erythropoietin or erythrocyte production rates) may be roughly characterized by its slope (sensitivity or gain) and by its normal operating point (i.e., the basal or threshold level of production rate)*. The effects of changes in sensitivity on controller response have been previously illustrated (see Figure 12). Figure 20 shows the effects on the bone marrow when both sensitivity (curve A) and operating point (curve B) are decreased from normal. Combinations of these cases, in which both sensitivity and operating points change simultaneously, can also be postulated. Dramatic changes in erythrocyte production and erythropoietin sensitivity of the marrow are possible by altering the shapes and position of the controller function. Erythropoietin production (as a function of tissue pO_2) is controlled in a similar manner.

The effect of a change in the bone marrow's normal operating point on erythropoietic activity was evaluated by model simulation (Figure 21). In this study, the parameter controlling the position of the basal production rate of red cells (P_1) was altered (see also Volume II, Figure B-3 for parameter definitions). Decrease in P_1 would be analogous to a primary anemia of bone marrow failure. The initial effect on production rate is dampened by the bone marrow transit time delay. The reduction in red cell mass (and hematocrit) accompanying this case produces a secondary response of erythropoietin release, which tends to partially restore production rate to a new, sub-basal, steady-state level. This process causes the undershoot of the simulated production rate. A long-term effect of this stress is also demonstrated, inasmuch as red cell mass does not appear to have stabilized even by sixty days.

The controller functions have not been well described in the human, and it is mostly from animal studies that their general characteristics are known.

*A linear function curve may be simply defined by its slope and intercept; a non-linear function curve requires other parameters for its characterization (as illustrated in Volume II, Figure B-3).

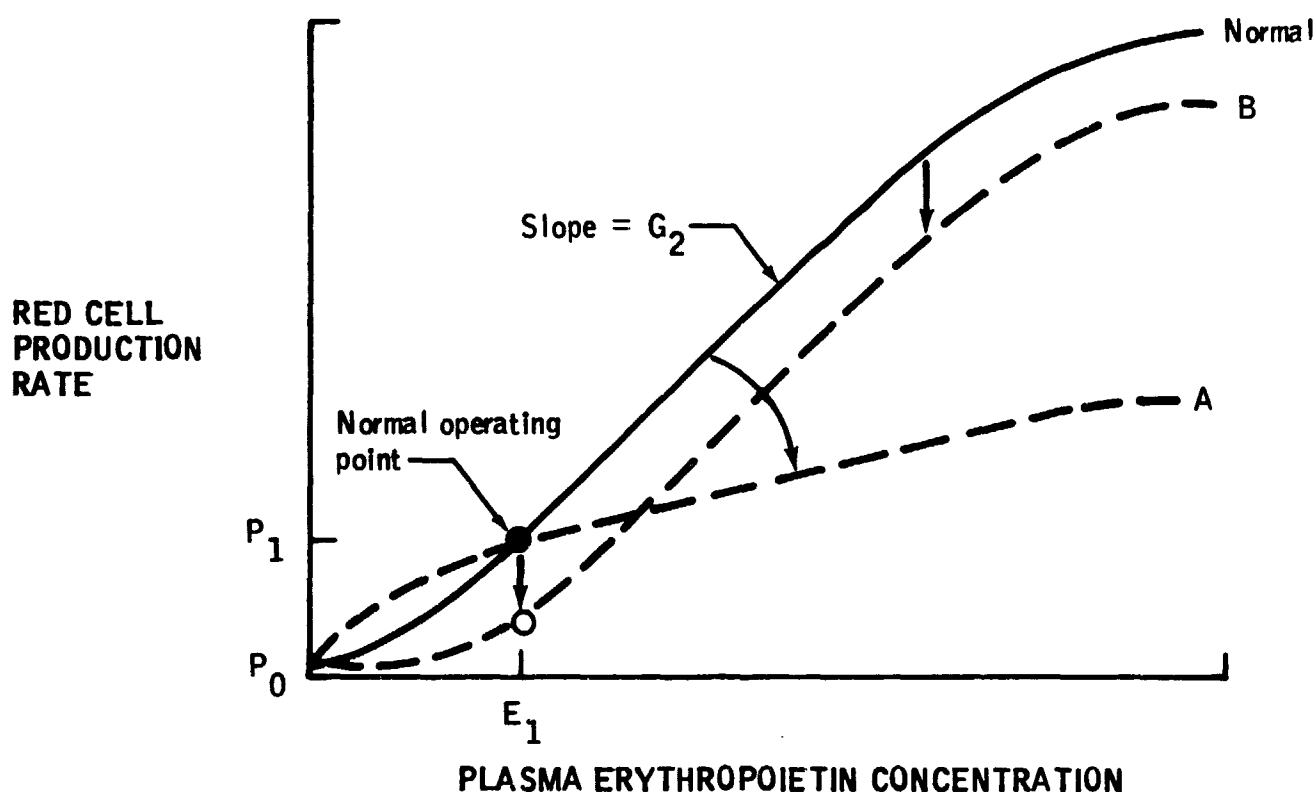


Figure 20: The erythropoiesis controller illustrating hypothetical alterations in bone marrow responsiveness. The normal function relating erythropoietin concentration to red cell production is shown as the solid curve. Two distinct types of controller shifts are indicated by the dashed curves. Curve (A): a simple reduction in slope (by rotation about the normal operating point) reduces the sensitivity (gain) of the marrow to erythropoietin while maintaining normal operating levels of red cell production. Curve (B): a downward shift in the normal function curve results in reduced erythrocyte producing activity for any given level of erythropoietin, although the sensitivity of the bone marrow to changes in erythropoietin are not significantly altered. P_1 = normal red cell production; E_1 = normal erythropoietin levels; P_0 = basal production rate.

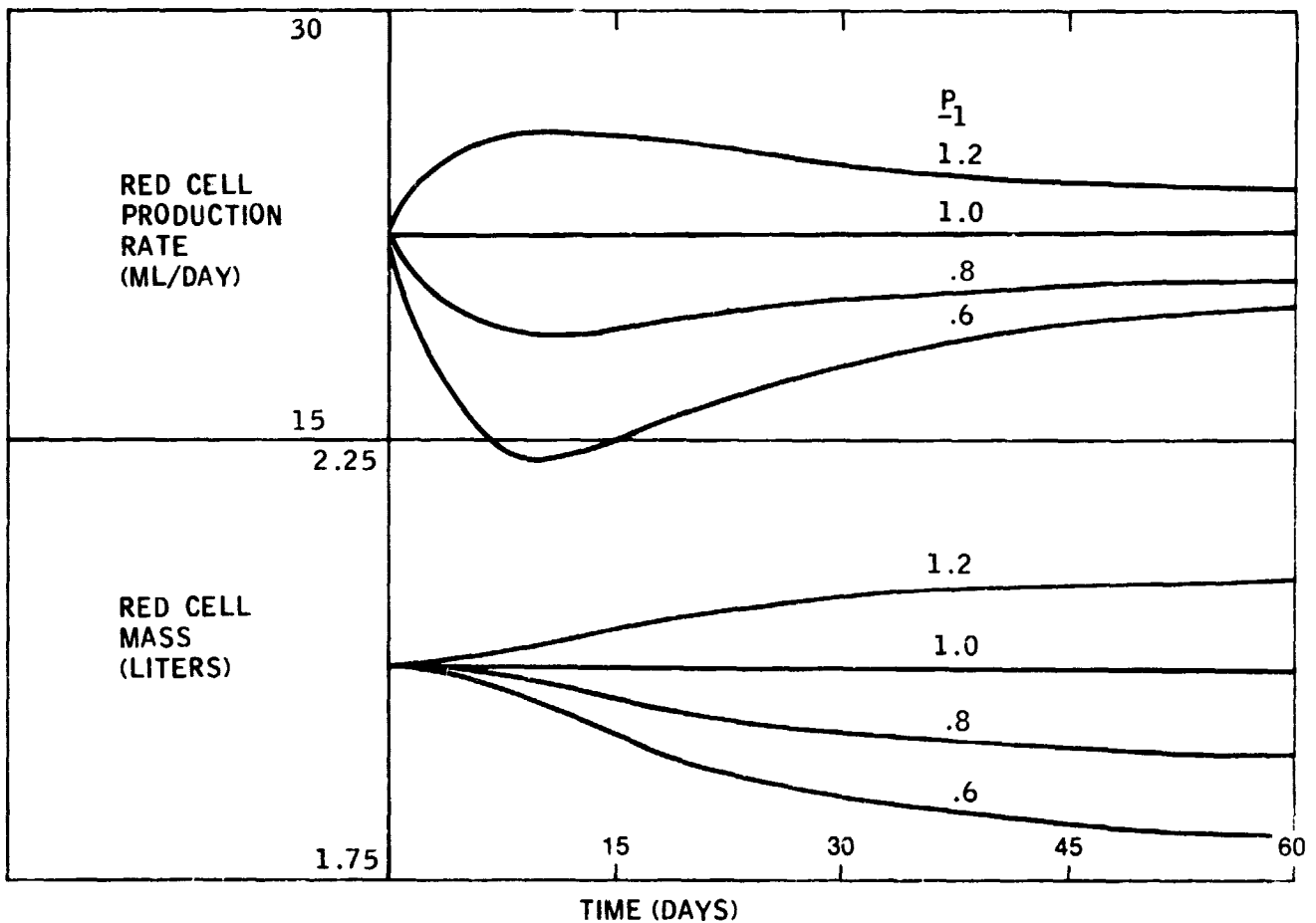


Figure 21: The simulated response to alterations in the operating point, P_1 . At zero time, the value of P_1 was adjusted from its normal value ($P_1 = 1$) to the value shown, and is maintained at that level for the period shown.

Comparison of simulation responses with experimental responses has verified the accuracy of the functions used in the model, and has permitted estimation of the parameters which characterize their shape and position. If these control functions behave similarly to those of other physiological systems, it may be assumed that their characteristic parameters may be altered during certain stressful conditions, particularly if the stress is maintained chronically. In fact, shifts in control function are one important way in which the body adapts to unusual conditions. There is a growing body of literature reporting specific instances of these types of adaptive changes. These reports have been reviewed in Volume II, and some are particularly relevant to the present study of space-flight anemia accompanied by hemoconcentration. It has been noted, for example, that hypertransfused animals become progressively more sensitive to erythropoietin within one to two days after transfusion of red cells (Schooley, 1965; Kretchmar, 1966). There is indirect evidence to suggest that prolonged decreases in erythropoietin concentration may lead to decreases in bone marrow sensitivity to erythropoietin (Gurney et al., 1961; Mylrea and Abbrecht, 1973). Others have shown that bone marrow responsiveness to exogenous erythropoietin decreases during restriction of food and water (Dunn and Lange, 1979; Giglio et al., 1979). If the size of the stem cell pool increases, as suggested during the response to hemolytic anemia (Erslev and Silver, 1975), this can be expressed in the model as an upward shift in operating point and an increase in sensitivity.

These findings, while limited, provide a rationale for testing the hypothesis that the renal or bone-marrow function curves are altered during space-flight. One preliminary model analysis suggested that space flight is accompanied by decreases and then increases in the controller operating point (Kimzey et al., 1976b), although the mechanism for inducing this change could not be postulated at the time. Since then, evidence has become available suggesting that negative energy and water balances (both characteristics of most space flights) could reduce bone marrow sensitivity and responsiveness to erythropoietin and thereby be responsible for a reduction in erythrocyte production. Evaluation of this hypothesis is discussed later in this section.

Red Cell Destruction

Red cell death normally occurs after about 120 days by extravascular hemolysis (i.e., sequestration and destruction of senescent red cells in the reticuloendothelial system of the spleen and liver), and, less commonly, by intravascular hemolysis. These pathways account for the normal attrition of about one percent of the circulating red cell mass per day. Abnormal loss of circulating red cells can occur by increasing the degree of hemolysis, and also by such factors as hemorrhage (including blood sampling losses) and splenic trapping. Radio-labeling techniques and analysis of body fluids for cell breakdown products are useful for determining if cell death is occurring prematurely.

Using these methods, a shortened red cell lifespan was detected for Gemini crewmen and astronauts on the 28-day Skylab mission. Toxicity resulting from a high pO_2 cabin atmosphere was probably responsible for the Gemini findings, but as yet, no cause for the Skylab results has been found. Table 10 lists a number of factors that have been examined to account for unusual red cell destruction. Factors that have been considered and ruled out include blood sampling, splenic trapping, exercise, and increases in cell membrane fragility. It is conceivable that excess hemolysis can be attributed to the stress of launch and reentry (high G forces and vibration) but a shortened postflight lifespan is not a consistent finding on all missions. Changes in red cell shape have been reported (Kimzey, 1977), but they have not been well correlated with the rates of red cell disappearance. However, since cell surface and shape changes are known to influence red cell survival, this mechanism probably deserves further attention. At present, it is not possible to distinguish between these various mechanisms of red cell disappearance using the computer model. A single parameter, the red cell lifespan, can, however, be used to test various hypotheses related to the rate, degree, and time course of destruction, whatever its origin.

It can be argued that the failure to measure a shortened lifespan does not rule out the possibility that excess hemolysis or removal of cells did occur to an extent that was beyond the resolution of the techniques employed. There are two such types of losses to consider: an acute loss, which may occur soon

TABLE 10
FACTORS PROPOSED FOR EXPLAINING INCREASED RED CELL DESTRUCTION
DURING SPACE FLIGHT

FACTOR	EVALUATION
Blood sampling	Ruled out on basis of ground control studies
Mechanical stress of launch and reentry	Cannot be ruled out; may be responsible for postulated age-dependent loss early in flight or decreased lifespan measured on 28-day mission
Change in red cell shape	Morphological changes were observed but were not correlated with red cell loss; may require further attention
Increased cell membrane fragility	Ruled out on basis of osmotic fragility tests
Exercise hemolysis	Ruled out on basis that increasing exercise in space was associated with less RCM loss
Splenic trapping	Ruled out on basis of spleen and liver scans on Apollo-Soyuz mission
Oxygen toxicity	Probable cause of hemolysis on Gemini and Apollo but not in Skylab

after launch, and a chronic, but low level loss which may continue for longer periods of time. With regard to the first of these, it has been proposed that an age-dependent loss of red cells cannot be ruled out (Johnson et al., 1977). In particular, the ^{14}C -glycine lifespan tests are based on a radio-label injected 30 days prior to flight, with subsequent measurement during the postflight period. Therefore, if red cells greater than 30 days of age at the time of launch were sequestered and destroyed selectively during the first few mission days, this event would not be seen in the ^{14}C -glycine survival curves. While such a process is conceivable, it must be consistent with observed levels of inflight hemoglobin concentration, inasmuch as any acute loss would lower those levels. Computer simulations that quantitatively evaluate this hypothesized route of loss will be presented later in this section.

The effect on red cell mass of chronic shortening of red cell lifespan is illustrated by the computer simulation of Figure 22 (solid line). In this study the normal lifespan was reduced by 10 percent (i.e., the approximate corrected change found for the crew of the 28-day mission as shown in Table 3), and the predicted effects during the subsequent 60 days were recorded. The model predicts a decrement in red cell mass of only several percent, reflecting the sensitivity of feedback compensation which limits red cell loss by increasing erythropoiesis (see also Figure 18). However, if production rates had not increased (or if they had decreased) in response to abnormal hemolysis, losses of red cell mass greater than 6 percent are predicted (see Figure 22 (dashed line)). This latter case may have been closer to the space-flight situation since erythropoiesis is believed to be inhibited in weightlessness. These simulations suggest that a large fraction of the total red cell mass loss observed on the 28-day mission may be attributed to a measured 10 percent decrease in lifespan assuming there is no compensatory increase in erythropoiesis. Smaller changes in lifespan may not be detectable (i.e., see control group in Table 3), but could still contribute significantly to the overall loss if the condition is maintained. In spite of the lack of extensive space-flight data, it appears reasonable to conclude that red cell destruction was not responsible for all of the measured losses of red cell mass, even on those missions where a decrease in lifespan was observed.

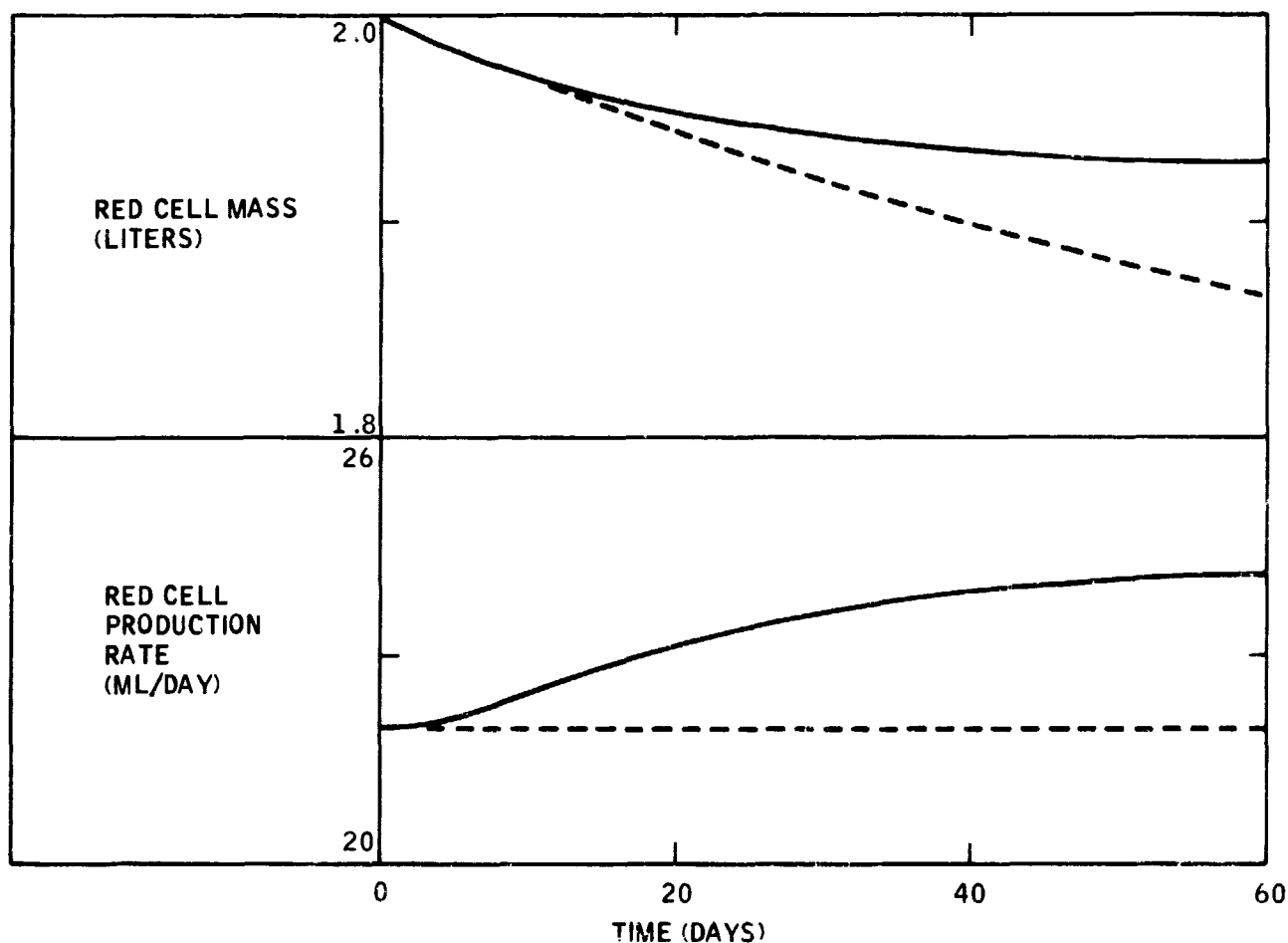


Figure 22: Simulation of the effects of hemolysis caused by reducing the red cell lifespan by 10 percent in the intact model (solid curves). A second case was considered (dashed line) where compensatory increases in red cell production are prohibited.

Differences Between Missions

Regardless of the cause of red cell mass loss during space flight, the problem of explaining the unexpected differences of these losses among the three Skylab missions remains. Figure 2 showed that the severity of red cell mass loss (as determined on the first recovery day after flight) was progressively smaller for each subsequent flight of longer duration. Since red cell mass was not measured inflight, it is only possible to speculate on inflight behavior. Two alternative hypotheses will be considered here; they will be termed the "regeneration theory" and the "continuous loss theory."

Regeneration Theory:

One hypothesis which was offered to explain red cell mass behavior claimed that regeneration of erythropoiesis occurs during prolonged space flight (Johnson et al., 1974; Kimzey, 1975; Kimzey, 1977). Accordingly, if the three missions are considered as a composite, a time-relationship between the red cell mass change and days following launch becomes apparent (Figure 7). These results suggest that "following some initial insult, during the first 2 or 3 weeks of flight, the red cell mass begins to recover, after a refractory period, at about 60 days" (Kimzey, 1977).

In order for the "regeneration hypothesis" to be credible, it must include an explanation for three events: the initial loss of red cells, the refractory period, and the inflight red cell mass-repletion process. With regard to the initial loss of cells, the preliminary belief was that it took place early in the flight due to either hemoconcentration-induced-hyperoxia (Leonard, 1974; Kimzey, 1977) or to splenic trapping of red cells older than 30 days (Johnson et al., 1977). The former mechanism is based on suppressed erythrocyte production, while the latter mechanism is effectively a form of red cell destruction. A refractory period, supposedly characterized by a red cell mass which temporarily stabilized at reduced levels prior to regeneration, was believed to be a result of continued hemoconcentration and decreased oxy-hemoglobin affinity. The only mechanism originally proposed for inflight regeneration of red cell mass was a transient hemodilution at about the same time that the red cell mass is theorized to begin rising toward control (Figure 23). The sudden drop in plasma hemoglobin concentration, if real, may have been due to a rise in plasma volume. It was hypothesized that this event

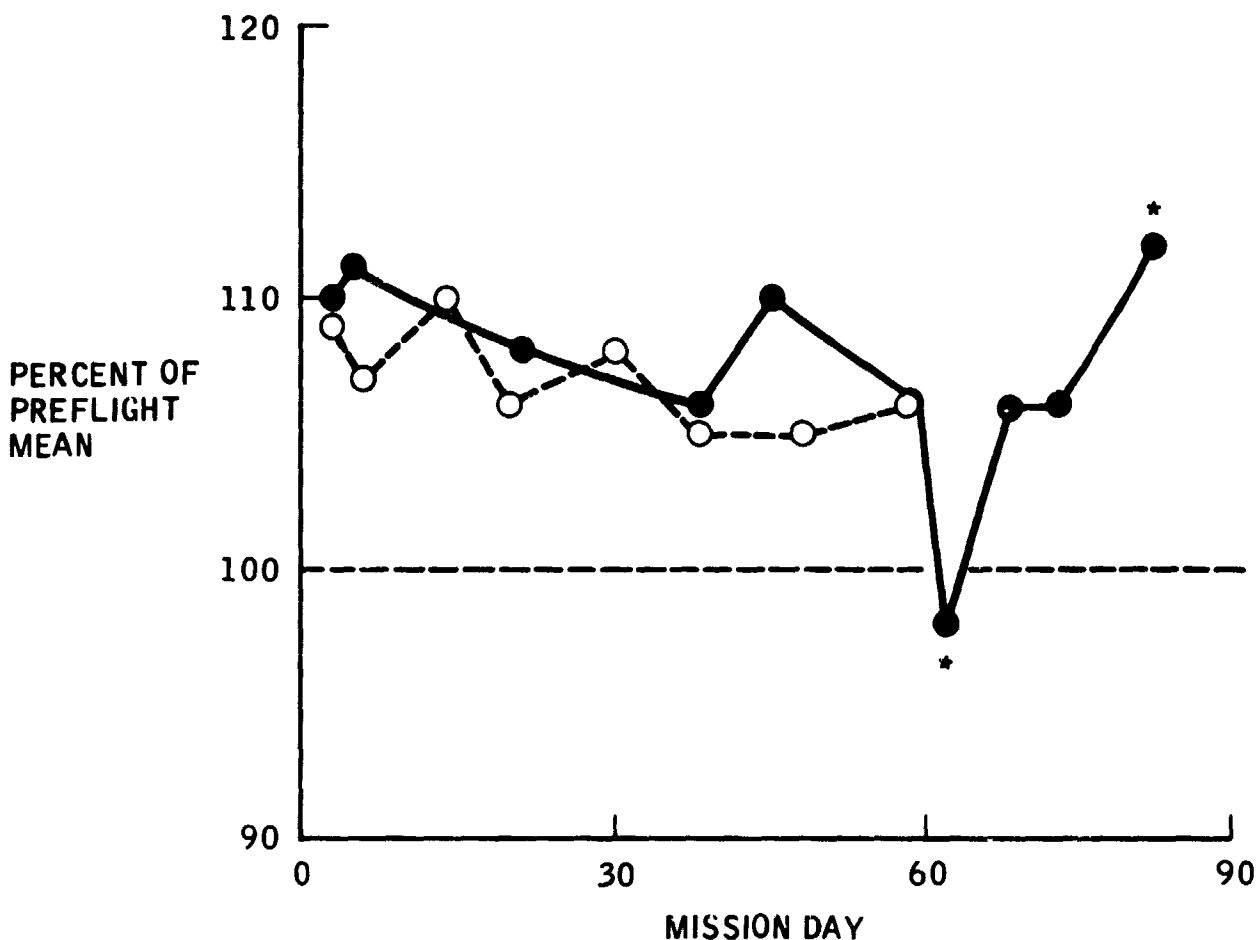


Figure 23: Inflight hemoglobin concentration during the 59-day and 84-day missions expressed as percent of preflight mean. The first determinations at Day 4 after launch show a 10 percent hemoconcentration reflecting plasma loss. Thereafter, hemoglobin levels decline slowly, presumably due to red cell mass loss. Each point is the mean of three crewmen. All data, except those marked (*) were determined by averaging measurements from two different methods (see "Skylab Findings"). The (*) data were obtained by the finger stick-hemoglobinometer technique only. This figure was obtained from preliminary Skylab reports (Kimzey, 1975), but the accuracy and meaningfulness of the (*) data is being reassessed (see text). The data in question were omitted from Fig. 5.

may have triggered the production of erythropoietin and red cells, and provide the basis for a regeneration phenomenon (Kimzey, 1977). The likelihood of regeneration late in flight was said to be supported by a post-mission reticulocytosis, which was apparent soon after recovery of the longest mission, but which did not appear after the shortest mission (see Table 4).

In spite of the attractiveness of the regeneration theory, it was based on assumptions that must be critically examined, particularly in the light of new analyses conducted in the interim period. The theory might be challenged on the following grounds:

- a) The regeneration curve of Figure 7 was constructed entirely from postflight measurements, but the implication is that they could reflect inflight red cell mass changes. Shipboard data collected on the first postflight day presumably reflects red cell mass prior to reentry. However, there is reason to believe that postflight recovery is not an accurate indicator of a postulated inflight recovery (see (b)).
- b) The kinetics of the postflight repletion response appears similar to that which occurs following any usual blood loss (i.e., hemorrhage, acute hemolysis, blood donation) and is likely controlled by similar mechanisms. Although these mechanisms are not completely understood, there is a good reason to suspect that hemodilutional anemia (caused by plasma refilling after blood loss) is a major causative factor in stimulating erythropoiesis (Adamson, 1968). Figure 7 suggests that regeneration during space flight is controlled by the same mechanisms that control postflight recovery. However, the inflight period is characterized (with the exception of one datum) by hemoconcentration (erythro-suppressing) in contrast to the hemodilution (erythro-activating) of the postflight period.
- c) Splenic trapping, one mechanism advanced in preliminary analysis to explain the original red cell mass loss, cannot be supported on the basis of spleen and liver radio-scans performed on the subsequent Apollo-Soyuz crew (Kimzey and Johnson, 1977). An age-dependent destruction of cells by other means cannot, however, be ruled out.

- d) While it is possible that a decreased oxy-hemoglobin affinity could contribute to oxygen unloading at the tissues and suppression of erythrocyte production, as proposed (Johnson et al., 1974), there is no strong evidence of this occurring either during space flight or bed rest. Furthermore, it is the conventional wisdom that a decreased oxy-hemoglobin affinity arises in response to tissue hypoxia and not the hyperoxia that was thought to be present (Swisher, 1974).
- e) A postflight refractory period in the recovery curves of the crew of the 28-day mission may have been caused by a lack of hemodilution as suggested by Kimzey (1977). However, an inflight refractory period cannot realistically be postulated based on postflight recovery kinetics. If, on the other hand, inflight hemoconcentration and a decreased oxy-hemoglobin affinity contribute to the postulated refractory period by inhibition of erythrocyte production, it is difficult to understand why the red cell mass does not continue to fall during this inflight period rather than stabilize.
- f) The mechanism by which regeneration was thought to take place centered on a single datum showing a sudden fall in hemoglobin concentration on day 60 of the 84-day mission (Figure 23). Closer inspection revealed that this value was heavily biased by measurements on one individual on a day when his water intake was unusually high. Also, an inflight technique of questionable accuracy was used (finger stick-hemoglobinometer method) to obtain that day's value in contrast to the other data of Figure 23, in which results from two techniques have been combined. It is unlikely that a short period of hemodilution, if it was real, could have caused a sustained period of erythropoietic activity leading to a 10 percent increase in red cell mass as suggested in Figure 7. In sum, it is questionable that hemodilution is a general occurrence during space flight and that it was responsible for inflight regeneration.

- g) A consequence of the regeneration theory is that initiation of red cell production is apparently independent of the presence or absence of gravity, but rather is a function of the time after first exposure to weightlessness (Kimzey, 1977). Absent from the hypothesis, if hemodilution is discounted for the reasons discussed above, is a suitable explanation of the processes which underlie these unusual kinetics, particularly the stimulus that causes regeneration to occur.
- h) The legitimacy of using data from different missions in the composite diagram of Figure 7 derives in part from the assumption that the conditions present during space flight which affect erythropoiesis were identical on each flight and that the primary difference between flights was their duration. However, it is known that at least two factors were present, diet and exercise, that were augmented on each subsequent flight. That is, increasing the duration of weightlessness was accompanied by increasing levels of diet and exercise. Both of these factors are known to affect erythropoiesis, as discussed earlier in this section. They also had a demonstrated effect on other tissues. It is generally agreed that the crew of the 84-day mission (on which red cell mass diminished the least) was successful in preventing significant muscular atrophy, circulatory deconditioning and weight loss by their superior exercise program and relatively hypercaloric diet (Thornton and Ord, 1977; Rummel et al., 1975).

The preceding comments are not meant to suggest that regeneration of red cells in space did not occur in some of the Skylab crewmembers. However, a plausible explanation for its occurrence is still lacking. While hemoconcentration is still highly attractive as a mechanism contributing to reducing red cell mass, it is not likely that the transient alterations in inflight hemoglobin levels can be invoked as a causative factor for regeneration. In summary, the available evidence does not appear to support regeneration as a general theory for erythropoietic activity during weightlessness.

Continuous Loss Theory:

An alternative, and more conservative, hypothesis that avoids most of the objections to the regeneration theory, is illustrated in Figure 24. It takes into account the idea that all missions were not identical. Therefore, a composite of flight data based on end-point determinations should not be used to describe inflight kinetics. The dashed lines in Figure 24 represent the decline in red cell mass during the inflight phase. While it is not possible to conclusively define the kinetics of this period, ground-based bed-rest studies suggest that a linear decline (as shown) may in fact have occurred.

If this is true, the rate of decline of red cell mass would be seen to be inversely related to the duration of flight. This is in contrast to the postflight recovery of red cells (denoted by solid lines in Figure 24), which appears to have been achieved at similar rates for all crews, except for the first two weeks of the shortest mission. This alternative theory can be termed the "continuous loss" hypothesis because it proposes that the loss of red cells is primarily a monotonic function throughout the inflight phase. While some regeneration cannot be ruled out, it would not be to the extent predicted by the regeneration theory as illustrated in Figure 7.

The challenge of the continuous loss theory is to explain how the loss rates of red cell mass could be different on each flight. Hemoconcentration is currently the most plausible factor implicated in suppressing red cell production, and was presumably similar on each flight. With this in mind, a rationale for the behavior described by the three curves of Figure 24 can be hypothesized. Specifically, it can be hypothesized that some erythro-suppressive phenomenon, common to all flights and a direct function of the weightless environment (perhaps hemoconcentration), acts to reduce red cell mass by inhibiting production, and that other factors that are different on each mission (e.g., level of caloric intake and/or exercise) modifies erythropoietic activity. Therefore, a varying behavior of erythrokinetics would be expected for each mission. In support of the continuous loss theory, are several recent findings that were not available during the immediate post-Skylab period. These are reported in the next three paragraphs.

First, a composite of bed-rest studies indicate a clear linear decline in red cell mass with no observable regeneration (Kimzey et al., 1979). However,

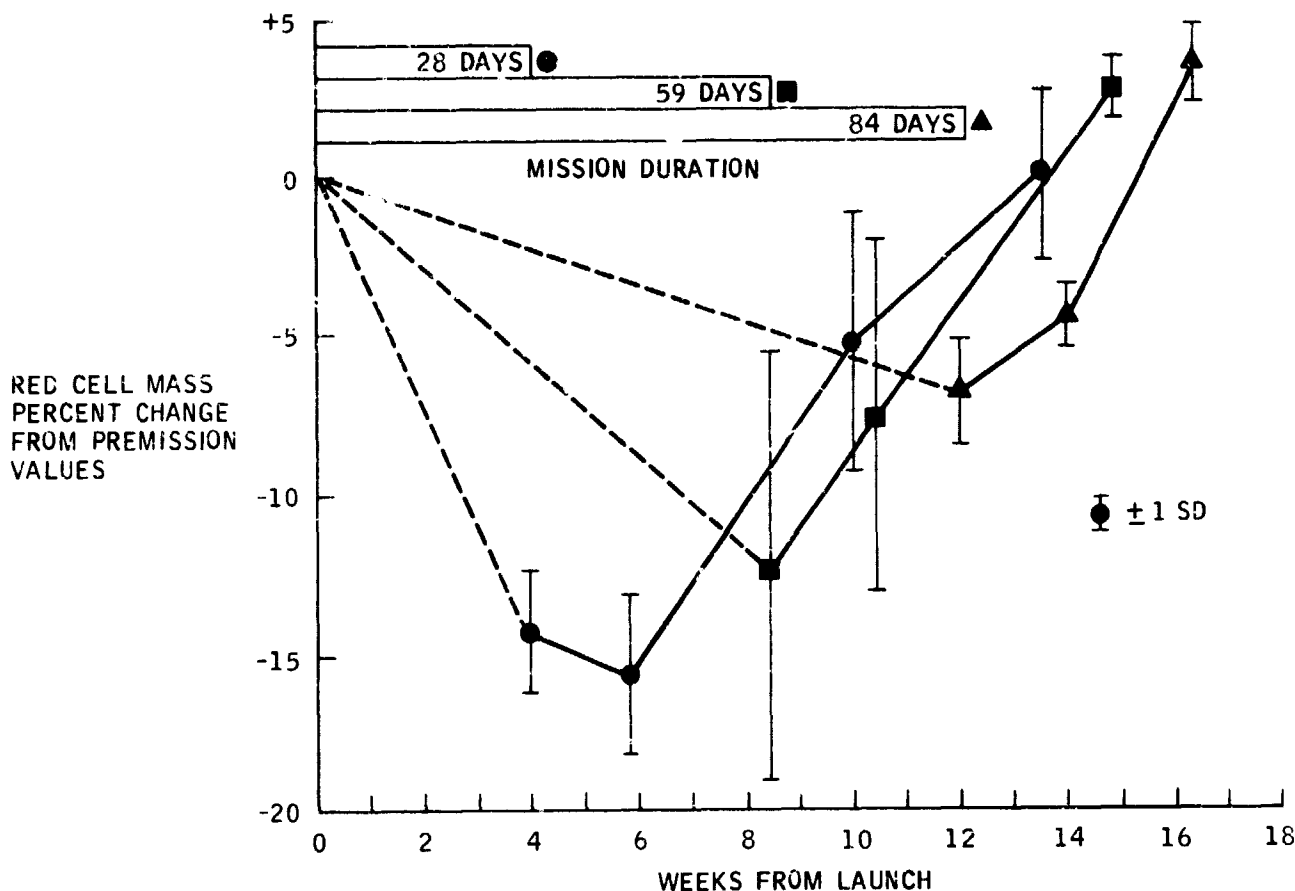


Figure 24: Changes in red cell mass in the Skylab crew measured from the day of launch. Each point represents the mean (\pm SD) change of the three-man crew as measured on the first day of recovery and during the subsequent postflight period. The dashed lines suggest the time course of inflight behavior while the solid lines connect the measured postflight values. The data shown here are identical to that shown in Fig. 7. Only the lines connecting the points have been altered to emphasize that each mission could be treated as a separate event rather than as points on a continuum. This representation of the data is termed the "continuous loss theory" in contrast to the "regeneration theory."

the regeneration theory of space flight requires the period of repletion to be delayed for up to two months after the hypogravic stimuli, and the bed-rest data includes measurements only up to 5 weeks (Kimzey et al., 1979).

Secondly, the studies reviewed in association with Figure 19 showed that both hemoconcentration and negative energy balance can be responsible for significant erythro-suppressive effects in mice. These animal studies are admittedly short-term experiments, but nevertheless, a negative energy balance was reported for the three crews, and this balance varied directly with red cell mass loss.

Thirdly, a similar trend for red cell mass among the three crews that is shown in Figure 24 was also reported for solid tissue loss (Leonard, 1982), and this was not thought to result from any adaptive or regenerative effects of zero-g exposure time, but rather from diet and exercise differences between the crews superimposed upon a common loss due to direct effects of weightlessness. The continuous loss theory becomes more attractive if it can be assumed that the degeneration of red cells in space behaves similarly to other body tissues.

Both theories can explain the different losses of red cell mass for the three missions; their basic dissimilarity lies in the postulated inflight kinetic behavior (monotonic vs. biphasic) of red cell mass and red cell production. Repletion of red cell mass would begin, according to the regeneration hypothesis, approximately two months after launch, whether or not the crew has returned to earth. In contrast, the continuous loss hypothesis postulates that red cell mass begins recovery only after the crew returns to earth and hemodilution accompanied by normal activity and diet occurs. The differences among the recovery rates of the three crews can perhaps be explained by the relative degree of hemodilution of these crews, in accord with the suggestion of Kimzey (1977). These and other important differences between the two theories are summarized in Table 11. Both theories were evaluated by computer simulation as discussed in the next section.

Data Limitations and Experimental Error

The credibility of any hypothesis or set of hypotheses is only as good as the data on which the interpretations are based. Most of the experimental

TABLE 11
COMPARISON OF TWO THEORIES TO EXPLAIN BEHAVIOR
OF RED CELL LOSS DURING SKYLAB MISSIONS

	<u>REGENERATION THEORY</u>	<u>CONTINUOUS LOSS THEORY</u>
1. Behavior of RCM Response	Loss curve of RCM is biphasic and proposes inflight recovery	Loss curve of RCM is monotonic
2. Comparison Between Missions	All Skylab missions considered identical except for duration	Hemoconcentration behavior assumed similar on all missions, but other modifying factors such as diet and exercise were different
3. Hemoconcentration	Hemoconcentration proposed as a causal factor in loss of RCM	Same
4. Inflight Hemodilution	Temporary inflight hemodilution responsible for regeneration	Temporary inflight hemodilution has little effect on long-term erythrokinetics
5. Refractory Period	Refractory period exists during second month inflight	No inflight refractory period proposed; delayed recovery of 28-day flight due to minimal postflight hemodilution
6. Recovery of RCM	Recovery of RCM initiated after two months from launch irrespective of time spent in space	Recovery of RCM initiated only after return from space

studies reviewed in this section, whether they be space-flight or ground-based, are deficient with regard to the spectrum of important hematological indices measured in the living system. For example, the computer model has identified the following parameters as being potentially critical to characterizing and interpreting the space-flight findings: hemoglobin concentration (or hematocrit), red cell mass, plasma volume, red cell production rate, red cell destruction rate, plasma erythropoietin levels, and blood P_{50} . Of less importance but equally as pertinent are arterial pO_2 , bone marrow sensitivity to erythropoietin, metabolic rate, and adequate control or monitoring of diet and exercise. Although techniques are available for their measurement in humans and animals, it is rare that more than several of these parameters are determined in any single study. In the Skylab program, for example, data for hemoglobin concentrations and monitoring of dietary intake were the only critical inflight measurements performed. Human bed-rest studies and animal studies include additional measurements, but an overall description of the erythropoietic response to hypogravity must still be based on a composite of many studies performed by different investigators under diverse conditions and on different animal species. The simulation model is ideally suited to integrate these data, but without additional experimentation, hypotheses cannot be suitably evaluated.

In addition to the lack of data, the job of interpreting and utilizing the data which are available is made difficult because of some inherent inaccuracies in traditional measurement techniques. Reference is made in particular to determination of erythropoietin, red cell production rates, and red cell lifespan studies; these are discussed here.

It is the current belief that red cell production is suppressed in association with reduced erythropoietin levels. An in vitro fetal mouse liver cell assay has recently been developed (Napier et al., 1977) that does claim to have the required sensitivity for measuring low level erythropoietin, and results using this technique are summarized later in this section. However, this technique is not yet in wide use, and other bioassay techniques currently available are generally not accurate for measuring sub-basal erythropoietin levels. Data are, therefore, extremely limited in this regard.

Red cell production rates are most accurately determined by the indirect method of radio-iron incorporation into the erythron. However, this method is

often neglected in favor of the more popular use of the reticulocyte index. Confusing results have been obtained during the post-space-flight and post-bed-rest periods in which high rates of reticulocytosis were not accompanied by simultaneous increases in red cell mass (Morse, 1967; Dunn et al., 1977). However, reticulocytes are not a reliable index of red cell production during periods of strong erythropoietic activity (Harris and Kellermeyer, 1970), so these data should be viewed with skepticism.

Red cell lifespan studies are used to determine red cell destruction rates and to ascertain the presence and degree of any hemolysis. Most of the procedures involve following the time course of disappearance of radio-labelled cells. Normally the tracking of these cells requires many days and if the subject is not in a hematologically stable state (production of cells balancing destruction), or if sequestration of cells occurs, the method yields inaccurate results. The changing red cell mass accompanying space-flight and bed-rest studies indicates a non-stable state and, therefore, caution must be used in interpreting the findings.

The simulation model can assist in identifying the accuracy with which various parameters should be determined. Sensitivity analyses and parameter variation studies indicate the relative importance of parameters and the degree to which changes in their values can influence overall hematological behavior. It has been found, for example, that shifts of oxygen-hemoglobin affinity can produce dramatic changes in tissue oxygenation and that measurements of P_{50} should be made with an accuracy better than ± 1 mmHg.

Comparison of model behavior with experimental observations and any resulting interpretation, as discussed in the next subsection, should take into account the limited data available and the experimental error associated with these data.

SIMULATIONS OF EXPERIMENTAL STUDIES

The model simulations that have been thus far presented have exemplified the behavior of the assumed theoretical model of erythropoiesis. However, they did not demonstrate the greater capabilities of the modeling approach that arise out of a more direct involvement with experimentation or experimental data. Model-to-date comparison is a much more challenging test of model credibility, and it affords the opportunity to interpret data and

guide the experimental process. In this section, a number of experimental studies that have been subjected to simulation analyses will be described.

These studies were performed using both human and animal subjects and involved both long and short-term events. Therefore, both the human and mouse versions of the erythropoietic control model were employed. The types of stresses which were examined can be divided into two major categories. In the first group are stresses which result in a relative polycythemia such as bed rest, thirst, dehydration, and space flight. Because of the scarcity of data concerning long-term relative polycythemia, it was believed that useful insights could be obtained by studying a second group of related stresses, that of absolute polycythemia, as exemplified by ex-hypoxic polycythemia and red cell infusions. All of these experimental maneuvers are relevant to space flight because they all result in hemoconcentration and erythro-suppression, factors identified early in this study as possibly being responsible for the red cell mass loss in space flight.

As the study progressed and the number of experiments that were simulated were increased, it became clear that factors other than hemoconcentration were involved, to an extent that remains to be determined. Additional hypotheses were therefore formulated, and these have been described individually in the previous section. In the following pages, the results of applying these hypotheses, both singularly and in combination, to produce realistic simulations of human and animal responses will be described.

Simulations of Altitude Hypoxia

A simulation analysis of altitude hypoxia is presented elsewhere (see Volume II) to demonstrate validity of the erythropoietic control model. One of the more interesting aspects of this simulation study turned out to be not the ascent to altitude, but rather the descent back to sea level. Subjects returning from prolonged stays at high altitude breathe air at ordinary oxygen levels, but their red cell mass and hematocrit are considerably elevated. In the absence of other changes that influence oxygen supply and demand, the model predicted that hemoconcentration would suppress erythropoietin and red cell production until the red cell mass and hematocrit returned to the original pre-altitude levels. Experimental data confirms these overall changes which the model predicts in ex-hypoxic subjects as illustrated in

Figure 29 (Dunn, Jarvis, and Napier, 1976; Jain et al., 1978; Huff et al., 1975; Buderer and Pace, 1972). However, it should be emphasized that a direct cause and effect between hemoconcentration and erythro-suppression has not yet been firmly established, although this seems to be plausible mechanism. If an increase in hematocrit resulting from a previous hypoxic exposure could lead to suppression of red cell production, it seemed reasonable to presume that the same type of suppression should occur if hematocrits were increased merely by infusing additional red cells. Analysis of this latter stress condition is described next.

Simulation of Red Cell Infusion

Polycythemia, induced by infusing red cells from a donor, has long been known to suppress erythropoietin and red cell production of the recipient (Gurney and Pan, 1958). Induction of the erythroid-suppressed state by this procedure in the mouse forms the basis of an erythropoietin bioassay. One of the few available studies of the long-term effects of red cell infusion in humans was reported by Birkhill et al., (1951), who showed that the suppression of red cell production was directly related to the amount of red cells infused.

A simulation of this experiment is illustrated in Figure 25. At the start of the simulation, 800 ml red cells were infused. The model was modified so that the infused red cells could be distinguished from the original circulating red cell mass and could disappear at a linear rate over a period of 126 days. Analysis of the model's response showed that the increased hematocrit leads to tissue hyperoxia, decreased release of erythropoietin, and suppression of red cell production. Loss of the recipient's red cell mass continues until the hematocrit decreases to a point where erythropoiesis returns toward normal and is equal to the destruction of recipient cells. As production rates continue to rise slightly (due to an hematocrit which is just below normal), the recipient's red cell mass reaches a minimum value and then rises toward its control level. The results suggest that erythropoiesis is under the control of hematocrit levels and the renal oxygen sensor in this instance is behaving as the "hemoglobinometer" described by Beutler (1969).

The simulation results compare favorably with the experimental results, both with regard to magnitude and time course of the changes in hematocrit, disappearance of infused blood cells, and suppression and recovery of the

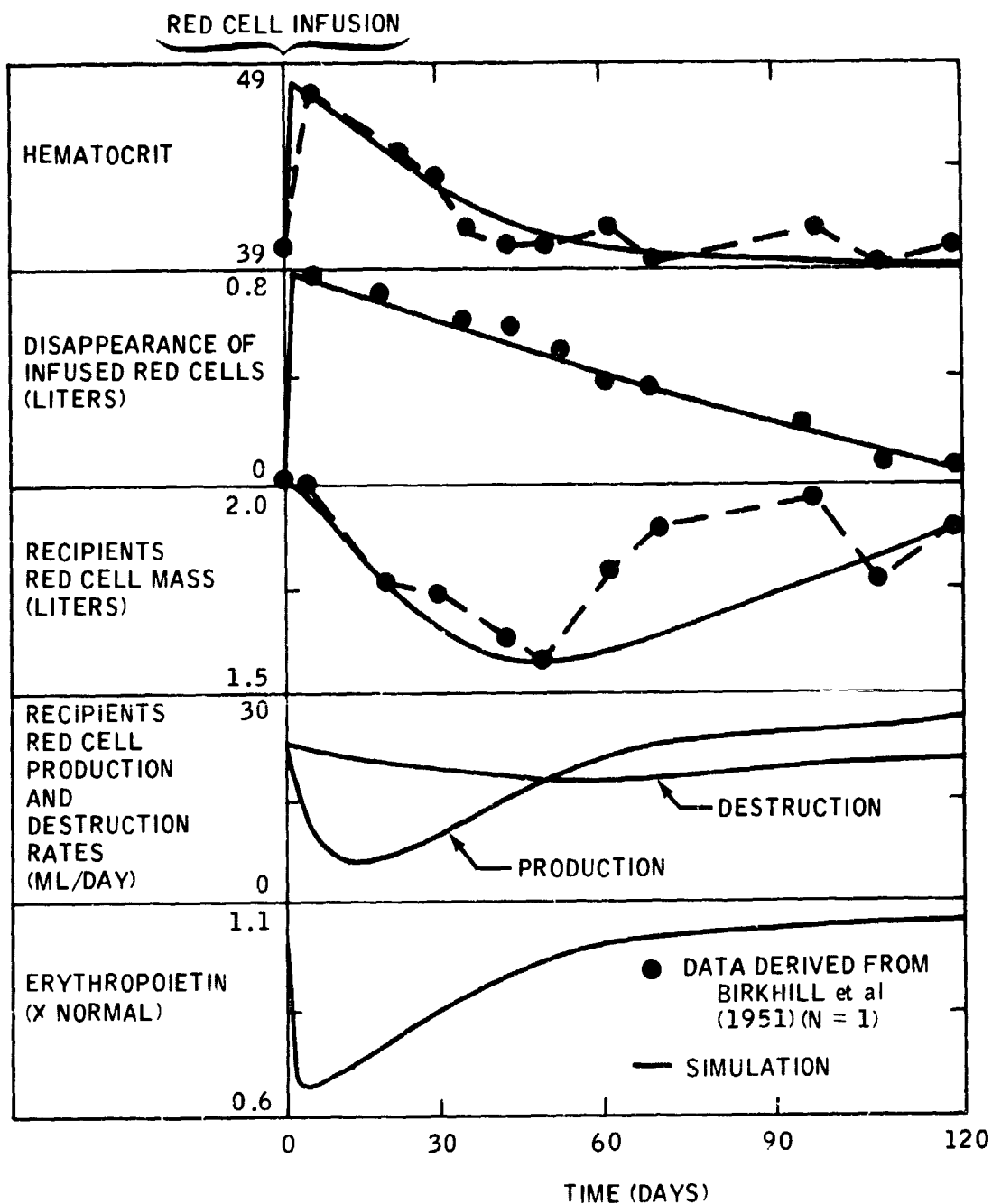


Figure 25: Simulated response to sudden infusion of 800 ml red blood cells (solid lines). Experimental data from a single subject are shown as filled circles and dashed lines (Birkhill et al., 1951). Infused cells disappear completely at a constant rate, while the recipients' red cell mass decreases to a minimum value as erythropoiesis is temporarily inhibited. Controller gain, G , was set to a value of 12 throughout the simulation.

recipient's red cell mass. Allowance should be made for the fact that the experimental study used only two subjects, did not have the accuracy that radio-labeled cells would have afforded, and was performed at a time prior to the identification of erythropoietin.

The similarity between the infusion response and the descent-from-altitude response of the previously described simulation should be apparent. In both cases, the increased hematocrit contributed to tissue hyperoxia and erythro-suppression. The degree of suppression was much greater for the descent phase than for red cell infusion, primarily because the initial hematocrit levels were higher in the former case. In both simulations, a similar value of controller gain ($G=12$) was used to obtain the best fit with experimental data. This also compared favorably with the controller gain value obtained from mice data previously reported by Hodgson (1970).

Bed-Rest Simulations

Bed rest and space flight are the only experimental maneuvers available at present for studying relative polycythemia in healthy subjects over long periods of time. The fact that these stresses have only recently come under experimental scrutiny undoubtedly is a major factor contributing to the lack of understanding of the present problem. Some of the general conclusions from bed rest and their relationship to space-flight hematology have been presented earlier (see "Bed Rest Findings"). In the following pages, a detailed analysis will be presented in which the mathematical model of erythropoiesis control was used to simulate and interpret findings from a 28-day bed-rest study (Johnson & Driscoll, 1977; Leonard, 1977; Kimzey et al., 1979).

The results of the 28-day Baylor College of Medicine (BCM) study have been presented previously in Figure 6. As in previous studies of this type, a small but significant decline of red cell mass (-7 percent) was observed. Various hypotheses were tested to provide insight into the response of the erythropoiesis system to bed rest. The principal hypothesis under consideration was that the red cell mass decrease during bed rest could be accounted for, in large part, by the observed downward shift in plasma volume. It was believed that the resulting hypovolemic polycythemia (i.e., relative erythrocytosis) would lead secondarily to suppression of erythrocyte production in the same manner as was suggested for the hypervolemic polycythemia (i.e., absolute erythrocytosis) of red cell infusion and post-altitude hypoxia.

The 28-day BCM study is particularly worthy of close scrutiny for several reasons. First, it was of the same length as the first Skylab mission during which a 14 percent decrease in red cell mass was observed. Secondly, the procedures used were nearly identical to those for the Skylab hematologic experiments and were performed under the same principal investigator. Furthermore, the time course was obtained for several important hematologic measurements which were not available from space-flight studies. These data are crucial for maximum utilization of the erythropoiesis model.

A preliminary simulation, performed with an earlier version of the computer model, showed reasonable agreement with a 35-day bed-rest study (Leonard, 1976). However, the data analyzed since that time, including the recent BCM studies, demonstrate that the red cell loss is a linear function with time (rather than the exponential disappearance predicted originally by the model or the biphasic regenerative behavior as suggested by the Skylab data) (Johnson and Driscoll, 1977). Secondly, the earlier observation by some investigators that red cell mass continues to decline following bed rest was confirmed during the 14-day and 28-day BCM studies (Figure 6). It appears that this "refractory" period can last for up to two weeks after bed rest is ended (Taylor et al., 1945; Miller et al., 1964; Morse, 1967; Hyatt, 1971). The red cell mass eventually returns to normal during the recovery phase, but the precise dynamics of this event are not clear because of the sparsity of data. The ability to mathematically simulate both of these phenomena, operative during the supine phase and the recovery phase of bed rest, was singled out as particularly desirable for the simulation study.

Details of the bed-rest study and of treatment of the data prior to simulation are provided elsewhere (Johnson and Driscoll, 1977; Kimzey et al., 1979). Simulations were performed using either the bed-rest hematocrit data as a driving function, or in some cases, using an idealized plasma volume function as a driver. Where possible, the simulation responses (i.e., red cell mass, plasma volume, erythropoietin, red cell production) were compared directly with other experimental data. In addition, other parameters were adjusted as necessary, in accord with postulated events in order to obtain closer agreement between model and observed responses. For convenience, the results will be divided into those related to the supine phase of bed rest, and the ambulatory recovery phase of bed rest.

Supine Phase of Bed Rest:

The simulation shown in Figure 26 was obtained using the experimentally determined hematocrit (from Figure 6) as the only model driving function. The overall gain of the feedback elements (i.e., G) was adjusted prior to the simulation until the predicted red cell mass at day 28 was equal to the measured value on that day. The gain was assumed to be constant throughout the supine and ambulatory phases, and its value was consistent with prior validation studies of the model involving descent from altitude and red cell infusions. The linear change in red cell mass and alterations in plasma volume predicted by the improved model are in excellent agreement with the measured values during the supine phase.

The model predicts an average fall in plasma erythropoietin levels of about 15 percent and in red cell production rate of about 27 percent during the first 28 days. During the subsequent recovery phase, both of these indices of erythropoietic activity increased above baseline levels. These model predictions are generally, but not totally, consistent with bed-rest data with respect to direction, magnitude, and time course of change (Morse, 1967; Scherba et al., 1975; Johnson and Driscoll, 1977). Reticulocyte counts (taken as a measure of red cell production rate) observed during bed rest, show either a 20 percent reduction (Morse, 1967), or no significant change (Dunn et al., 1977). The only measurements of erythropoietin made during bed rest showed a small decrease which was not statistically significant (Dunn et al., 1977) to confirm a decline in erythropoietin (Dunn et al., 1977). However, there is some question that the assay used was sufficiently sensitive to identify decrements from normal levels as it seemed to be in measuring increments in erythropoietin. In addition, post-bed-rest elevations in erythropoietin and reticulocytes have been reported (Dunn et al., 1977) and were generally consistent with the model results of Figure 26.

This portion of the simulation suggests very strongly that hematocrit changes alone can account for the magnitude and time course of the red cell mass decrements observed during the bed-rest study. In addition, the non-linear plasma volume loss and the time delays in bone marrow production were important contributory factors in producing the linear nature of the red cell mass decrease in the model and possibly in the human subjects as well. The effects of these factors on the dynamics of red cell loss are shown more clearly in Figure 27. The three simulations were accomplished by reducing

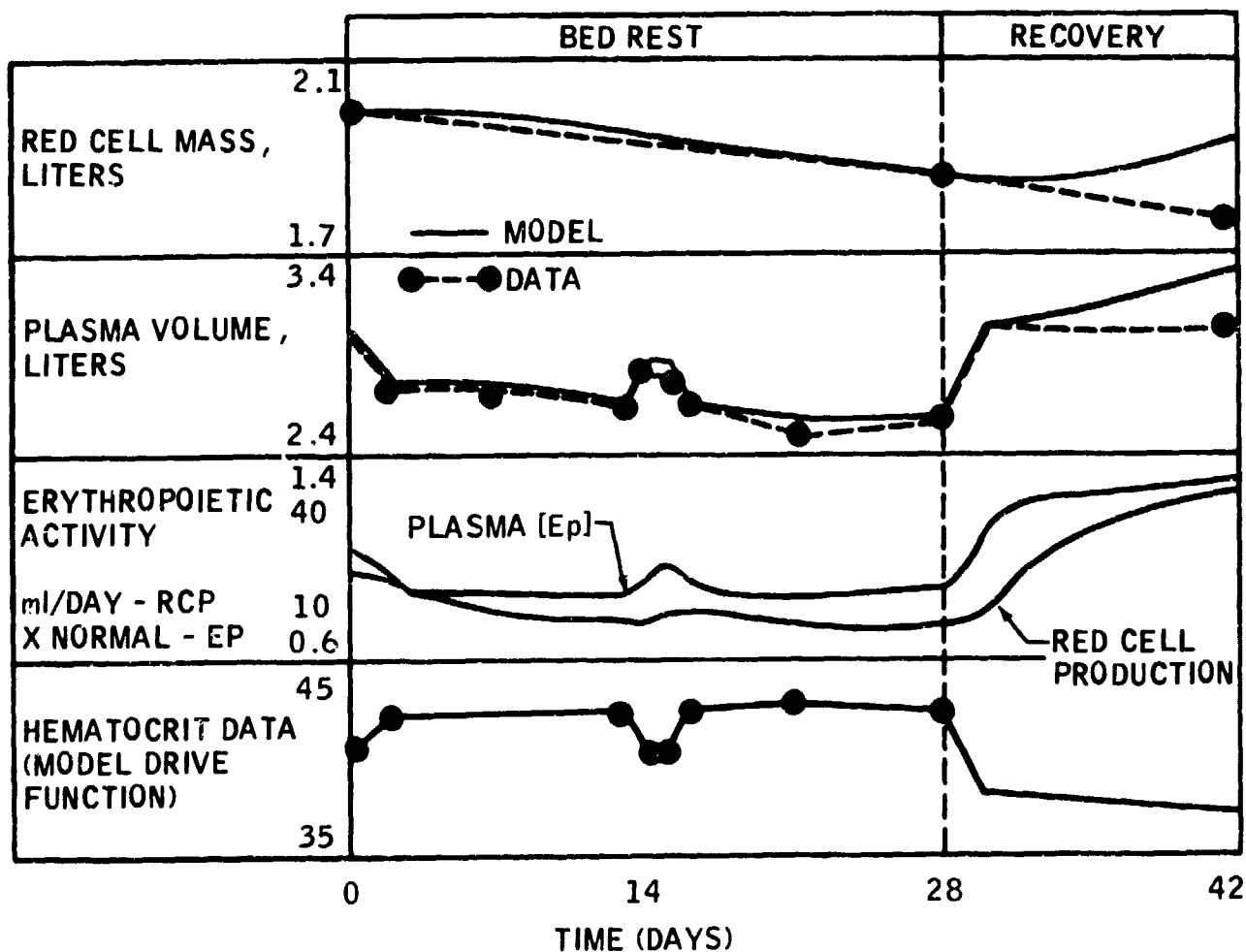


Figure 26: Simulation of the Baylor College of Medicine 28-day bed-rest study. Hematocrit data was used to drive the model during bed rest and recovery phases. Dashed lines and solid circles represent experimental data (Johnson and Driscoll, 1977). The off-normal transient at 14 days is a result of ingestion of saline (see caption in Fig. 6). It appears to have affected erythropoietic activity to a small extent, but the long-term effect on red cell mass seems negligible. Controller gain, G , was set to 10 throughout the simulation.

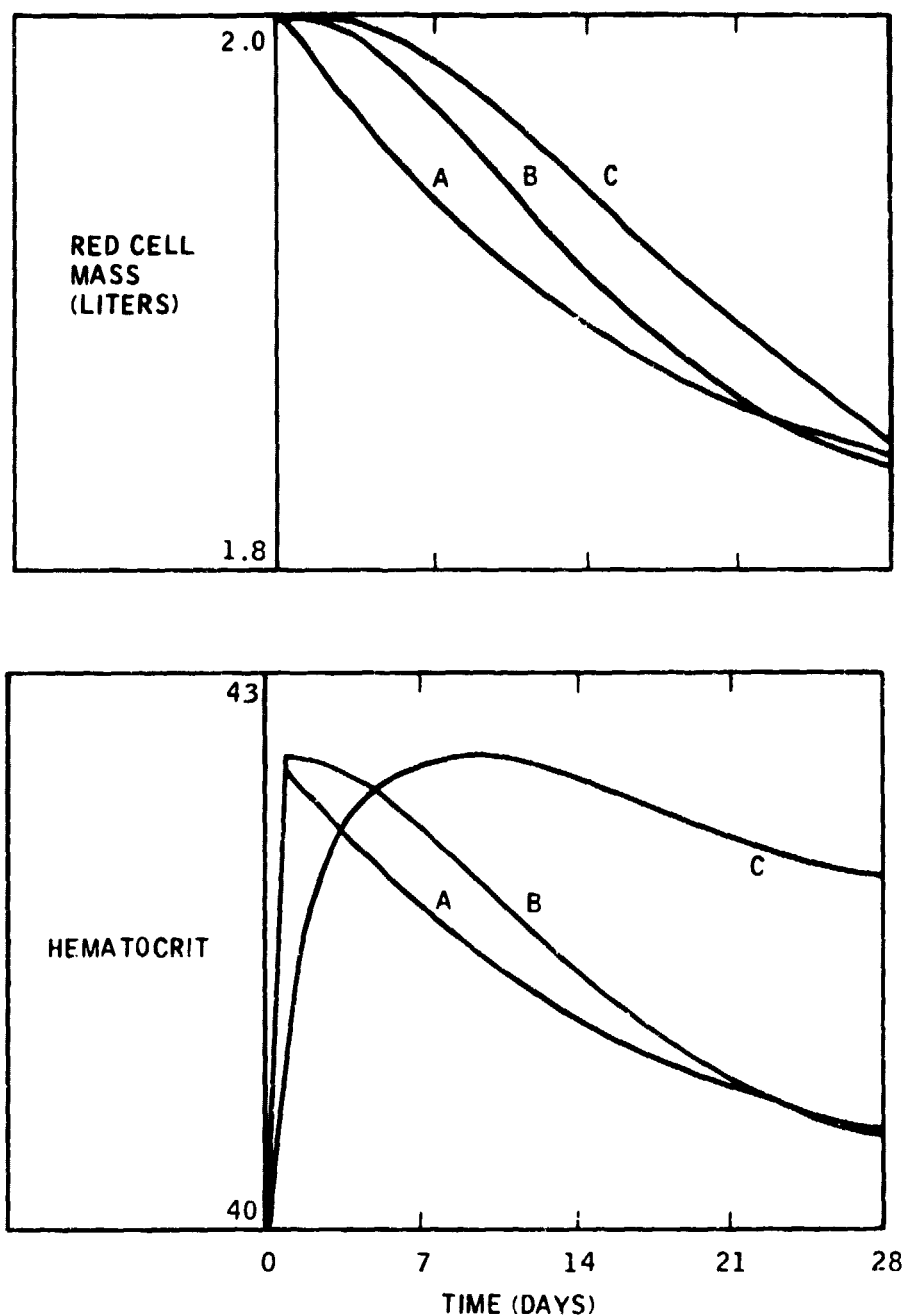


Figure 27: Effect of bone marrow time delay and dynamics of plasma volume (PV) disappearance on red cell mass and hemoconcentration behavior during a bed-rest simulation. (A) 300 ml PV step decrease using model with time delays set to zero, (B) 300 ml PV step decrease using model with normal first-order time delay of 4 days, (C) PV decreases exponentially with 300 ml depleted at end of two days and 200 ml additional loss thereafter, using model with normal time delay. Gain of model adjusted in each case to provide the same total red cell loss at the end of 28 days.

plasma volume to the same extent in each case. Using only an idealized step decrease in plasma volume and no time delays, the exponential decline of curve A is generated. The addition of the four-day bone marrow transit time delay increases the linearity of the red cell mass response as shown in curve B. Curve C represents the optimal predicted response and was produced by assuming a more realistic exponential decline of plasma volume and leaving the time delay function intact. Therefore, the only difference between curves B and C is a result of the differences between the assumptions regarding the dynamic behavior of plasma volume loss. In case C, the hematocrit declines relatively slowly compared to the other cases, in spite of an equivalent decrement in red cell mass. This occurs because the plasma volume continues to decrease throughout the four-week period in case C. It is noteworthy that the model's responses became more accurate as more realistic features were added.

Bed-rest studies to date have shown that red cell mass decreases linearly, without apparent limit, up to at least 35 days. These simulations have replicated this phenomenon and in the process, have revealed that this is true only as long as plasma volume continues to decline. If the plasma volume stabilizes at a reduced level (Figure 15), the hematocrit will fall with continued red cell loss and the depressant effect on erythropoiesis will be diminished. This process will continue until red cell production converges toward the now slightly reduced destruction rate (if lifespan of RBC is constant, destruction rates in the model are proportional to the total mass of circulating red cell mass). A new equilibrium will be established at which time the red cell mass will be reduced, but it will neither increase or decreases and hematocrit will be slightly above normal (a steady-state error of this type is expected for proportional control systems). This regulation of red cell mass represents a normal physiological feedback process. As can be visualized from Figure 28, this process can take a considerable length of time for perfect equilibrium to be established.

Recovery Phase of Bed Rest:

The initial model simulation, driven by experimental hematocrit data, failed to predict the continued decline in red cell mass which was observed during the 14 days following the end of bed rest. As shown in Figure 26, the post-bed-rest simulation (repeated in Figure 29 (panel A)), exhibits a delay in red cell mass recovery for only several days, a result of the bone marrow

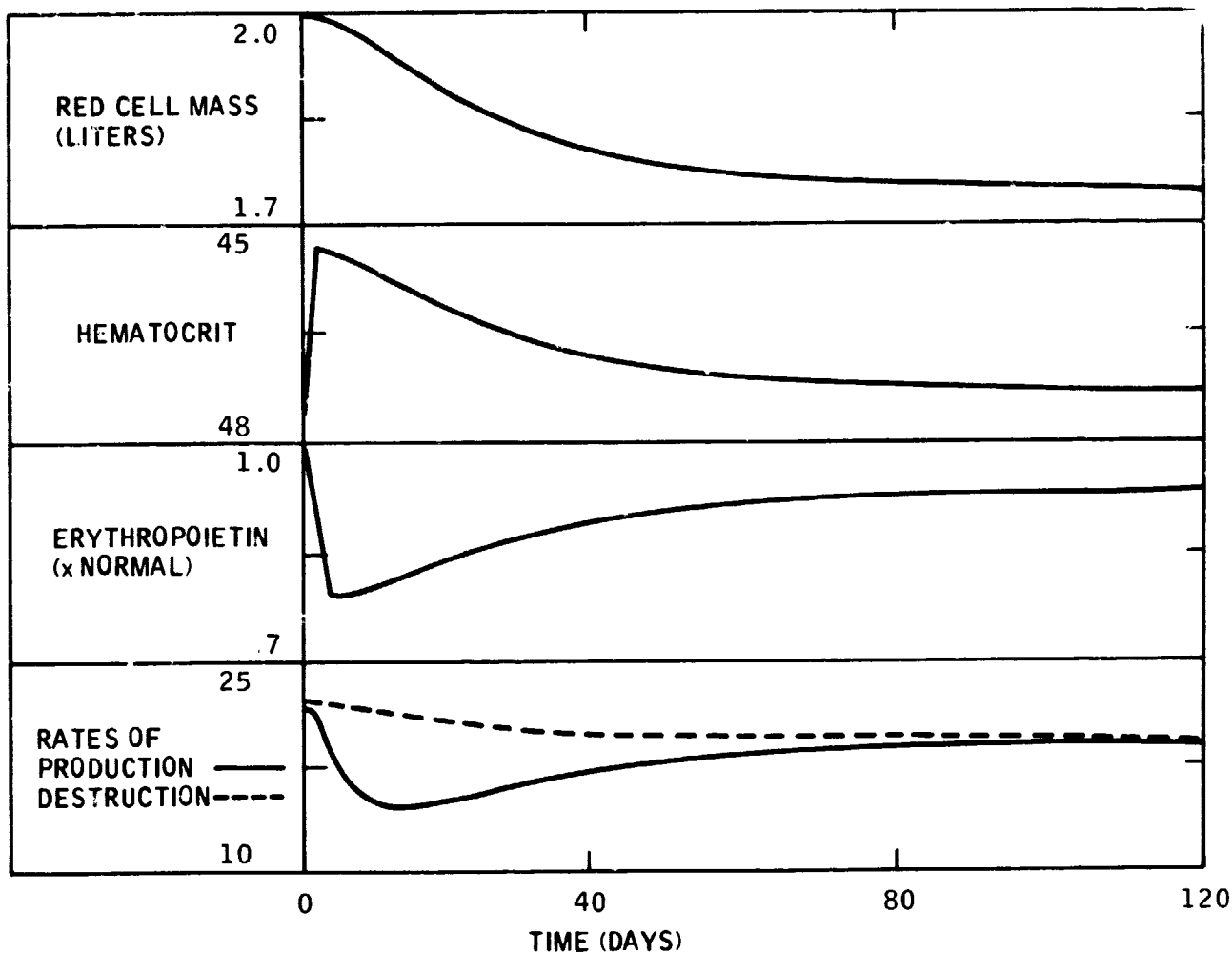


Figure 28: Simulation response to a step decrease (300 ml) of plasma volume showing approach toward equilibrium. The entire response is due to a hemo-concentration effect. The simulation suggests the self-limiting nature of red cell loss when plasma volume losses stabilize. Equilibrium is realized when red cell production and destruction converge and become equal.

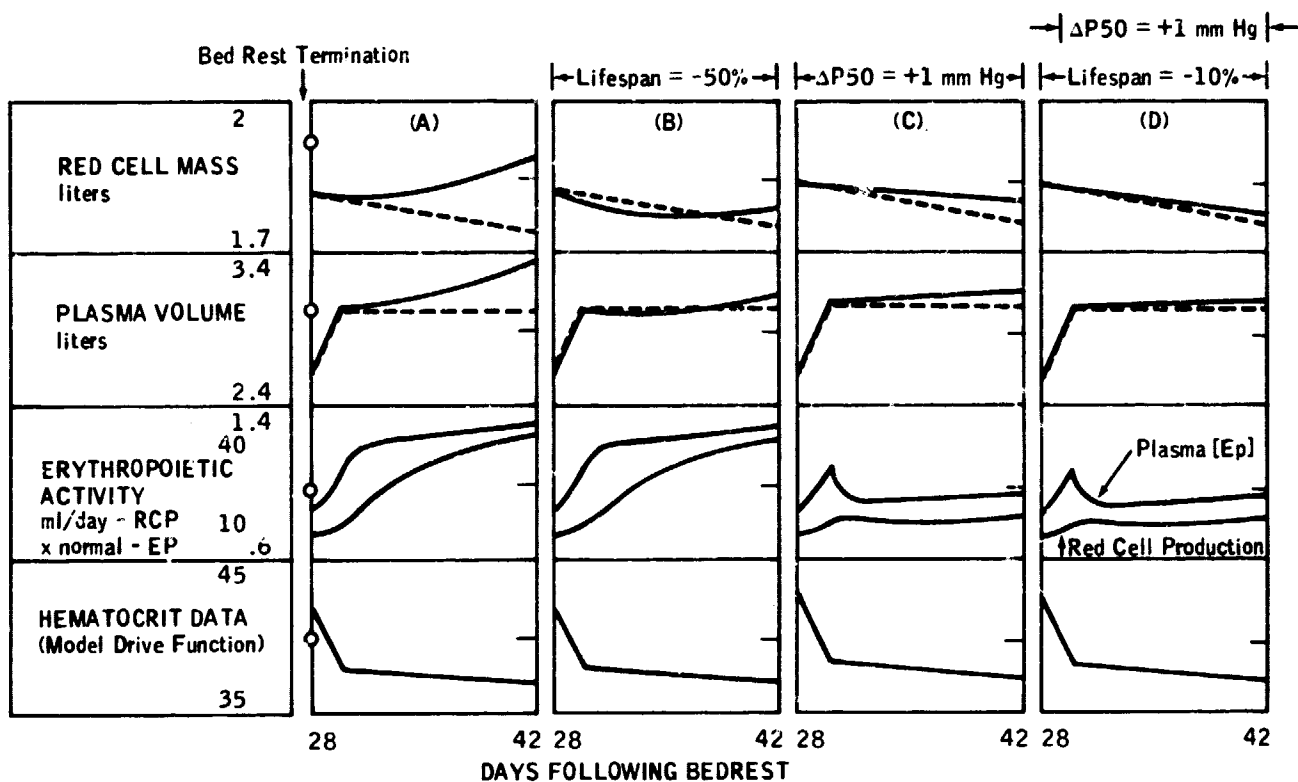


Figure 29: Simulation of various hypotheses to account for delayed recovery of red cell mass during post-bed rest period. The pre-bed rest control values are shown by solid circles in panel (A). The supine phase of bed rest which precedes the recovery period is given in Fig. 26. The hematocrit data were used to drive the simulation in all cases shown. (A) effect of hematocrit alone, (B) effect of hematocrit, and 50 percent reduction in red cell lifespan, (C) effect of hematocrit, and P50 shift, (D) effect of hematocrit, lifespan reduction and P50 shift combined. Dashed lines are experimental data (Johnson and Driscoll, 1977).

delay factor, which is included in the model. The model was used to examine hypotheses that could account for the delayed recovery of red cell mass for the remaining post-bed-rest period. Three specific post-bed-rest hypotheses were tested: that plasma volume returns toward control levels more slowly than was assumed (not shown), that there was a temporary increase in the rate of red cell destruction (panel B), and that there was a shift in oxygen-hemoglobin affinity in response to the modest hemodilution at recovery (panel C). These will be discussed next.

The sparsity of data for the period immediately following bed rest brings into question the true rate of recovery of the post-bed-rest plasma volume and hematocrit. No measurements of plasma volume or hematocrit were determined until two weeks after the BCM bed-rest study. Since red cell production has been shown to be sensitive to hematocrit shifts, it was reasonable to test the hypothesis that plasma values at recovery do not return toward normal as rapidly as had been assumed in the simulation of Figure 29(A). When the assumption that two weeks were required to return plasma volume to normal (instead of the two days previously assumed) was tested, the simulated red cell mass response during the first recovery week was substantially improved; that is, there was a slower replacement of red cell mass. While it is improbable that plasma volume recovery was this slow (Morse, 1967; Johnson et al., 1971), this simulation suggests that a more complete experimental determination of the recovery response is desirable. Further demonstration of the importance of plasma volume and hematocrit on recovery dynamics will be found in the section devoted to space-flight simulations.

The model was used to test the hypothesis that decreased exercise levels during bed rest conserves fragile red cells which are more vulnerable to destruction during the early ambulatory period. This hypothesis was tested by reducing the mean red cell lifespan in the model during the recovery period in order to represent the more rapid destruction of fragile cells. The results (see Figure 29, panel (B)) indicated that in order to completely account for the recovery response, the mean cell lifespan had to be increased to values which appear to be unrealistically high (i.e., 50 percent reduction in lifespan throughout a 2-week period).

Although evidence is not available to confirm changes in blood P_{50} during bed rest or space flight, it is interesting to speculate what effect such a shift might have had on the time course of red cell mass recovery from these

stresses. Based on findings showing decreased oxy-hemoglobin affinity associated with many forms of hypoxic disturbances (Brewer, 1974), it was postulated that increases in P_{50} could have occurred during the recovery from bed rest in compensation for the observed hemodilutional anemia. The simulation results suggested that only a very small increase in P_{50} of about 1 mmHg would account for a large part of the decreased red cell mass during the two weeks of recovery (i.e., compare panels (A) and (C) in Figure 29). Erythropoietin release is suppressed along with red cell production. The transient increase in erythropoietin concentration shown in Figure 29(c) at the time the P_{50} shift was activated corresponds to a similar event reported during the 28-day bed rest (Dunn et al., 1977). The profound influence that small changes of P_{50} had on the response was surprising, although suppressed erythropoietin is not an unexpected result (Beutler, 1969).

The possibility was considered that more than one mechanism may be simultaneously operative during the recovery phase. For example, reductions in red cell lifespan and increases in P_{50} could have occurred which were small enough to be experimentally undetectable, but which could produce measurable effects on red cell mass over a long enough period. This hypothesis was used in the simulation shown in Figure 29 (D) where the lifespan was decreased 10 percent in combination with a +1 mmHg shift in P_{50} . This resulted in improved agreement with red cell mass response although red cell production levels failed to rise above control.

A peculiarity noted in at least two bed-rest studies (Morse, 1967; Johnson and Driscoll, 1977) was an expected sharp increase in reticulocyte index after bed rest, in the face of an unexpected continued decline of red cell mass. Morse (1967) speculated that this paradox could be accounted for by a "compensated hemolytic syndrome in which the rate of production balances the rate of hemolysis." This is in accord with the recovery simulation of Figure 29 (B), if one accepts large values of red cell destruction. Ineffective erythropoiesis, a mechanism not included in the model, will also produce reticulocytosis while circulating red cell mass is reduced. However, there is no strong evidence to suggest significant incidences of ineffective erythropoiesis (Johnson and Driscoll, 1977), and only sparse data exist supporting hemolytic events (Morse, 1967). A possibility that remains to be examined is that red cell production during recovery is much lower than the reticulocyte index indicates. Under conditions of strong erythropoietic

stimuli, it is known that reticulocytes appear much earlier than usual and are not a reliable index of red cell production (Harris and Kellermeyer, 1970). This effect is not accounted for in the model. Other hypotheses for the delay in recovery of red cell mass include hemorrhage, splenic sequestering, and effects of blood sampling, but these have tended to be ruled out (Johnson and Driscoll, 1977; Kimzey, 1975).

The results presented thus far support the hypothesis that red cell losses during long-term supine bed rest, red cell infusions, and descent from high altitude are all normal physiological feedback processes in response to the sequence: hemoconcentration, enhanced tissue-oxygenation, suppression of red cell production. This is not to suggest that other mechanisms do not play a contributory role, but merely that the data available do not permit one to distinguish between alternate theories. There have been other hypotheses proposed to account for the suppression of the erythropoietic system during bed rest, and these have included: a reduction in oxygen requirements (Shcherba et al., 1975; Shvets and Portugalov, 1976; Morse, 1967); age-dependent loss of red cells (Kimzey, 1975); an increase in plasma phosphates (Johnson et al., 1974); increased renal blood flow (Leonard, 1974; Fuller et al., 1970; Shcherba et al., 1975); changes in gain and thresholds of the kidney-bone marrow controller (Kimzey et al., 1976); and changes in energy balance (Dunn and Lange, 1979). Some of these factors will be evaluated in the following simulations of ground-based animal studies and human space-flight studies.

Dehydrated Mouse Simulation

In the previous simulations, similar responses were demonstrated for cases involving both an absolute as well as a relative polycythemia. That is, the model was unable to distinguish between a hemoconcentration-induced-erythro-suppression caused on the one hand by an excess of red cells, or on the other hand by a loss of plasma volume. Also, this similarity was not in opposition to the available experimental data. However, more recent studies using dehydrated mice as a potential experimental model of space flight, have suggested some previously unrecognized differences between absolute and relative polycythemia. Because of the potential importance of these findings to the simulation analysis task, a collaborative effort was initiated between the scientific investigators of the animal experiments and the systems

analysis team. The results of this integrative approach provided clues that factors in addition to hemoconcentration may be involved in the space-flight and bed-rest responses.

The experiments which will be discussed have been described in detail elsewhere (Dunn, 1978; Dunn and Lange, 1979a, 1979b; Dunn, Leonard, and Kimzey, in preparation; Dunn, 1980), and the results are summarized in Figure 30. Briefly, dehydration in mice by water restriction produces a 22 percent reduction in plasma volume within the first 24 hours, and a 39 percent reduction within 72 hours. The proportional elevation in hematocrit was accompanied by suppression of erythrocyte production rates, and by the end of three days, a noticeable reduction in red cell mass. A decrease in red cell production (determined by radio-iron incorporation into newly formed cells) was measured on each of the first three days of dehydration, with suppression becoming progressively more severe with time. This trend was also evidenced by a decrease in cellularity of hemopoietic tissue. These findings could have been expected in light of the mechanisms previously discussed, involving tissue hyperoxia and suppression of a humoral regulator, erythropoietin. Erythropoietin titers were reduced, as expected on the second and third days of dehydration, but surprisingly, erythropoietin levels were normal on the first day. This apparent temporary dissociation between erythropoietin and red cell production was partially resolved by the finding that the splenic tissue became less sensitive to erythropoietin within 24 hours after dehydration. (In mice, the spleen is an important erythropoietic organ.) The reduced sensitivity was attributed to a reduced food intake which is found to be a voluntary response to water restriction. In addition, a small reduction in oxygen consumption was noted by the third day of dehydration, which may have been related to the loss in body weight. It was, therefore, hypothesized that a food restriction-negative energy balance can suppress erythropoiesis by direct effects on the hemopoietic tissue and independent of the hemoconcentrating effects of dehydration (see "Effect of Diet", this section, and Figure 19).

By comparing the responses of dehydrated, food restricted mice to those of red cell-transfused mice, it was possible to separate the effects of hemoconcentration (which occurs in both groups) from that of food restriction (which occurs only in the former group). This is indicated in Figure 31 where suppression of red cell production is observed for both dehydrated mice and

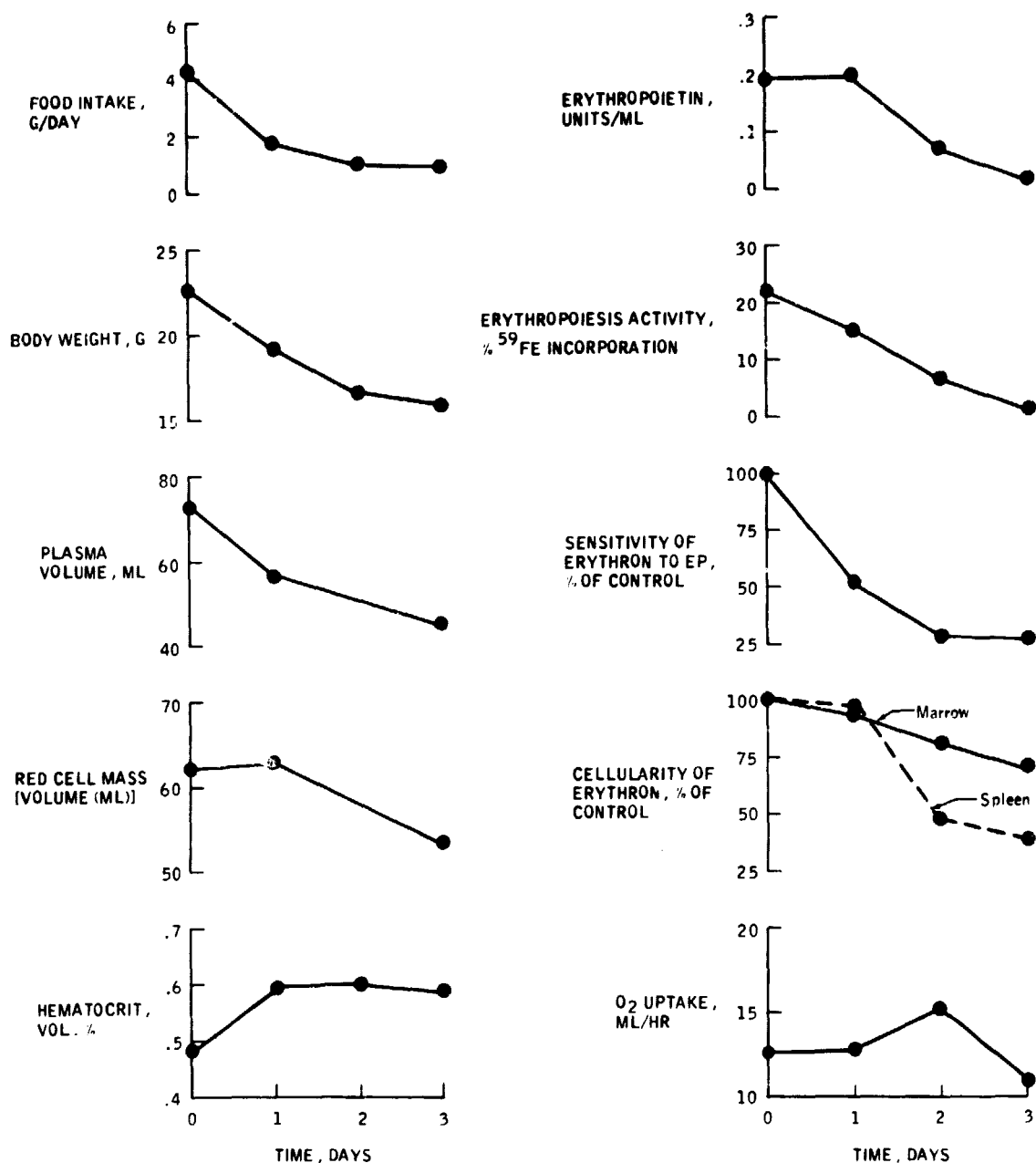


Figure 30: Response to dehydration in mice restricted from water for 3 days. Loss of body water is reflected by reduced body weight, plasma volume, and increased hematocrit. The voluntary food restriction that always accompanies water restriction in mice results in further weight loss and presumably influences changes in splenic sensitivity to erythropoietin, cellularity of the erythron, and oxygen uptake. A reduction in erythropoietin, erythropoiesis, and red cell mass are the secondary hematological responses to food and water restriction as suggested by the hypothesis chart of Fig. 19. (Data obtained from studies of C.D.R. Dunn and co-workers, 1978, 1979b, 1980).

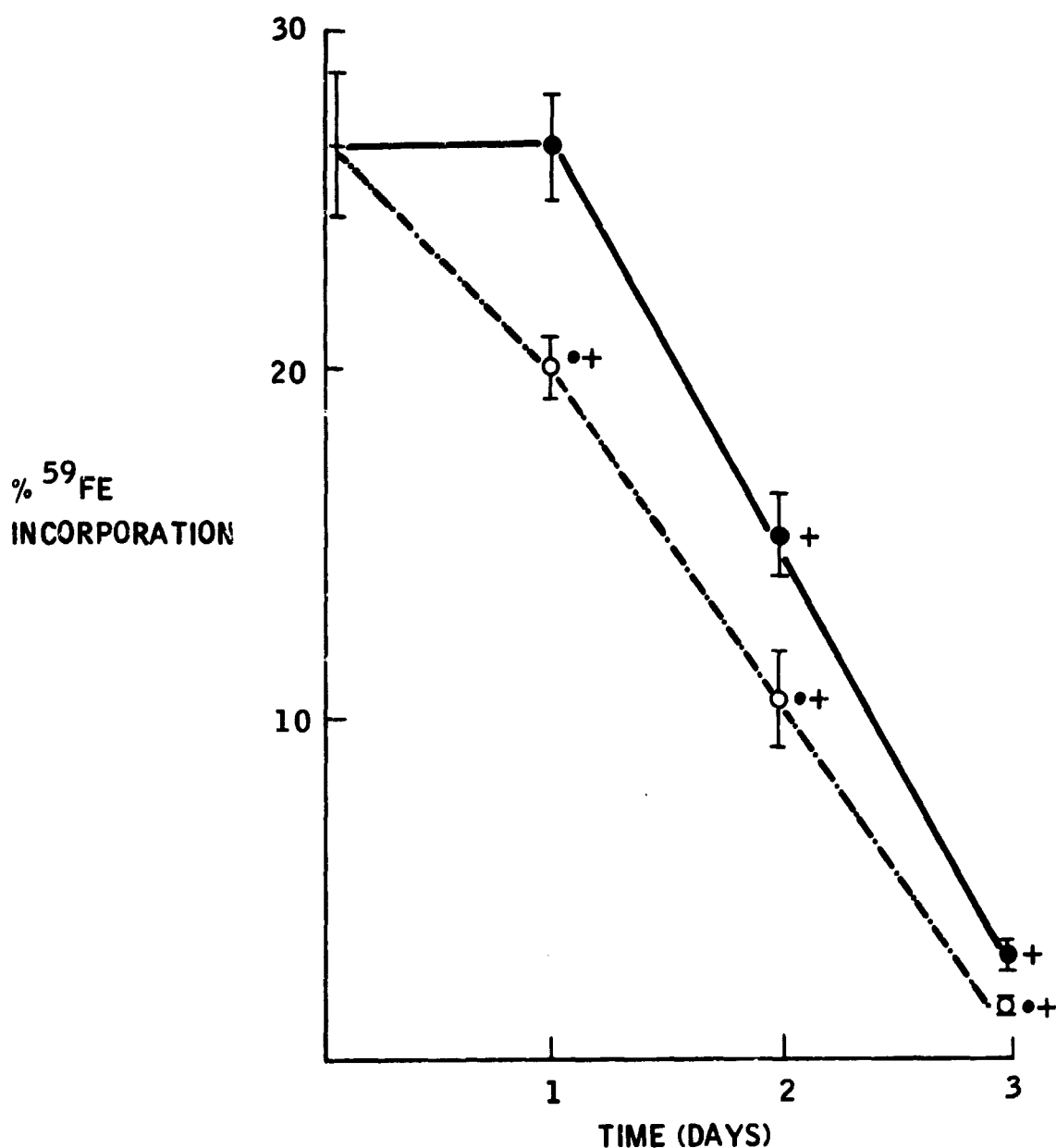


Figure 31: Rates of erythropoiesis in dehydrated (open circles, dashed line) and transfused (closed circles, solid line) mice. Vertical bars indicate \pm SEM. (* = $P < 0.05$ from controls; + = $P < 0.05$ of dehydrated mice compared to transfused animal.) Since the transfused mice ate and drank normally and the dehydrated mice were also food restricted, it is assumed that the difference between the two curves reflects erythroid suppression due to an energy related component.

mice transfused to a hematocrit similar to that observed in dehydrated animals. Assuming that the hemoconcentration-related component is similar for both groups, the differences between the two responses can be taken as a reflection of the energy related (i.e., food restricted) component. These results suggest that the initial suppression of erythropoiesis in dehydrated mice (i.e., within the first 24 hours) is entirely due to a reduced food intake. Thereafter, suppression can be attributed equally to the hemoconcentration and negative energy balance.

These findings, while demonstrating that factors other than hemoconcentration are important, did not reveal the precise pathways which are operative. For example, the failure of erythropoietin to become suppressed during the first day in spite of significant hemoconcentration was not explained. Other studies suggested that hemoconcentration (of ex-hypoxic mice) is capable of suppressing erythropoietin within several hours (Dunn, Jarvis, and Napier, 1976). In addition, other important factors were not considered in interpreting the results, such as the effect of hypovolemia and hypervolemia during relative and absolute polycythemia, respectively. Blood volume is an indirect modifying influence on oxygen transport as described earlier (see "Effect of Hematocrit Levels on Oxygen Transport.") Nevertheless, the wealth of data from these animal experiments suggested that a more rigorous examination of the data using systems analysis techniques might help resolve the major issues.

An understanding of the experimental results is facilitated by a theoretical analysis of the differing effects of hemoconcentration by either increasing the red cell mass for example, by hypertransfusion, or by reducing the plasma volume, for example, by dehydration (see Figure 32). Both conditions are characterized by hemoconcentration with resulting increases in oxygen delivery, decreased erythropoietin, and suppressed erythropoiesis. The increased viscosity accompanying hemoconcentration provides a resistance to blood flow and leads to an erythro-suppressive force which opposes, but apparently does not overwhelm the direct hemoconcentration effect. Several important differences also exist between the two hemoconcentration stresses: a) dehydration is hypovolemic while transfusion is hypervolemic; therefore, the effect of blood volume on blood flow (i.e., the oxygen delivery rate) is additive to the viscosity effect during dehydration, but in opposition during

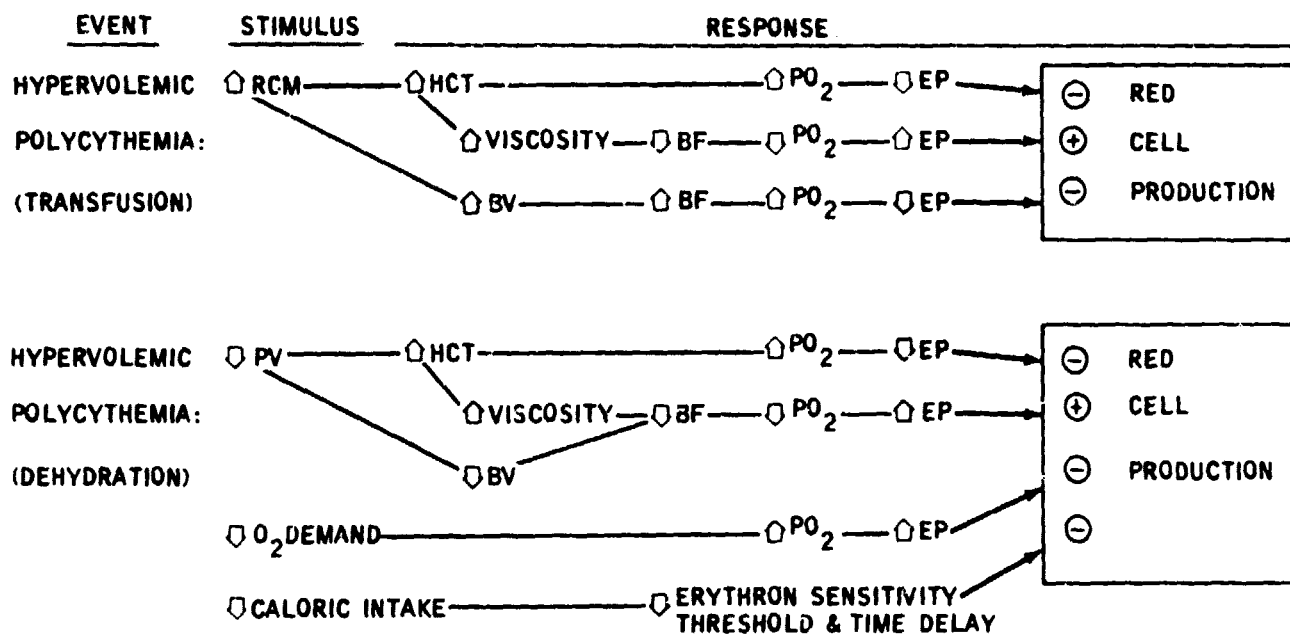


Figure 32: Hypotheses to explain differences between transfusion and dehydration polycythemia. (PV = plasma volume, RCM = red cell mass, HCT = hematocrit, BV = blood volume, BF = blood flow, PO₂ = renal tissue oxygen tension, EP = plasma erythropoietin concentration \oplus = increase, \ominus = decrease).

transfusion; b) dehydration in mice results in a decreased oxygen demand which could have a significant effect if manifested at the renal oxygen sensing site; c) dehydration in mice also has been found to be accompanied by reduced ad libitum dietary intake, and this may be responsible for the reduced responsiveness and a decreased time delay in cell maturation observed in hemopoietic tissue. The pathways by which diet, and especially a negative energy balance, might affect erythrocyte production was previously discussed in detail (see "Effect of Diet" and Figure 19). The crucial fact here is that diet restriction can suppress erythrocytes directly, without altering erythropoietin.

The individual effects of these pathways have been studied by model simulation techniques and those analyses suggest that red cell mass would be expected to decrease more rapidly and to a larger extent with dehydration (accompanied by food restriction), than with transfusion.

Simulations of the three-day dehydration experiments were performed in a version of the erythropoietic control model which was specifically adapted to the mouse. This version of the model is described by Leonard (1978) and Nordheim et al., (1982). The elements of the original human model which were modified to formulate a mouse model included parameters that were based on size (i.e., blood volume, plasma volume, red cell mass, and total oxygen uptake), and parameters that were based on values which are known to be species-specific, irrespective of size (i.e., red cell lifespan and the oxygen-hemoglobin equilibrium curve). In addition, validation studies were required to ascertain if the model could realistically respond to short-term stress conditions as it does to longer term disturbances. One such validation study is presented in Figure 33, in which the erythrocyte production responses to single and multiple injections of erythropoietin are illustrated. These results compare favorably to the dynamic behavior of reticulocytosis following erythropoietin injections, as measured some years ago in the mouse (Gurney et al., 1961). These and other short-term validation studies (Dunn, 1978) provided the basis to utilize the model for simulating dehydration and infusion experiments.

Two simulations based on the analysis of Figure 32 are shown in Figure 34. Identical increases in hematocrit were accomplished by either step increases in red cell mass simulating transfusion or by more gradual reductions in plasma

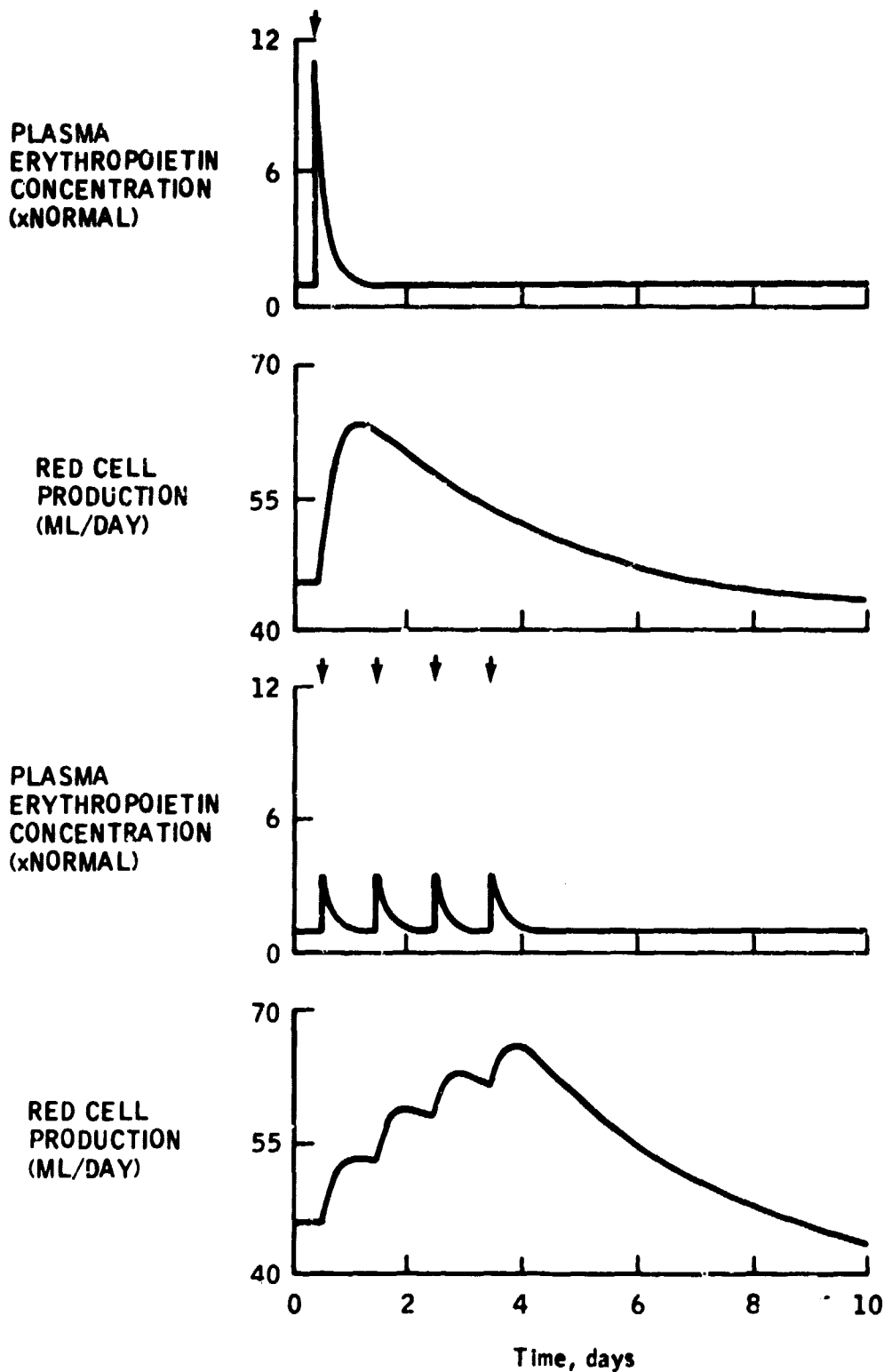


Figure 33: Simulated responses of "mouse" model to erythropoietin injections. (A) bone marrow response to single dose of erythropoietin, (B) bone marrow response to multiple doses of erythropoietin. Arrows indicate time of injections.

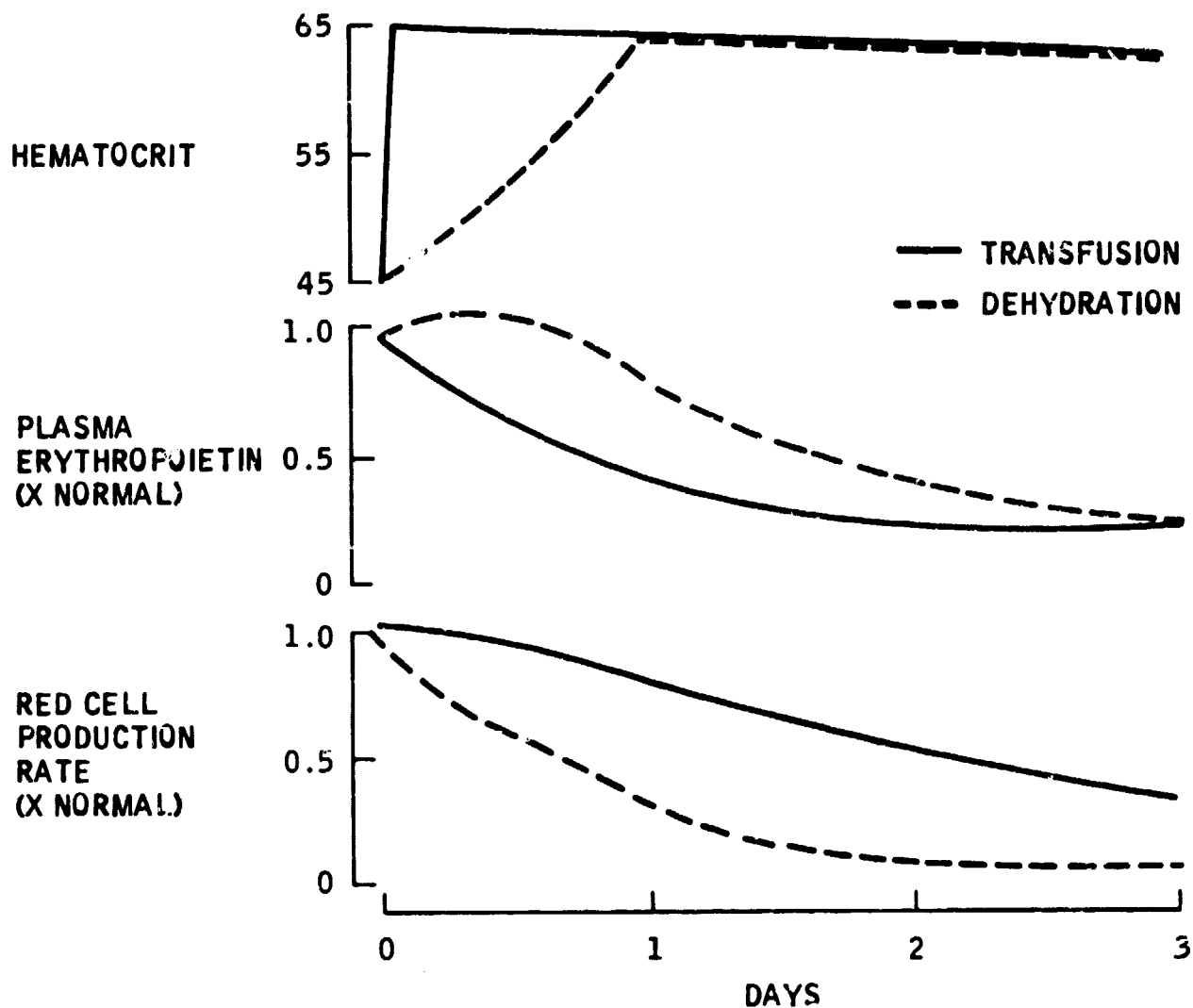


Figure 34: Simulated responses to red cell transfusion (solid line) and dehydration (dashed line) using "mouse" model. For the case of dehydration, the plasma volume was reduced over a period of one day while in the case of transfusion red cells were infused instantaneously, resulting in a similar degree of hemoconcentration for both cases. In addition, the following parameter adjustments were made during the dehydration simulation only: red cell maturation time reduced from 2 days to 0.5 days; controller operating point, P_1 , reduced from 1.0 to 0.2; blood flow reduced to 90 percent of control for the first day and then returned to normal. Controller gain $G = 18$, for both simulations.

volume similar to the dehydration maneuver. The transfusion response, which agrees with animal (Figure 31) and human (Figure 25) studies was produced by using only the influence of hemoconcentration. If hematocrit changes alone were the only factors considered in the dehydration case, its responses would be extremely similar to the transfusion responses because the model responds to the magnitudes of the hematocrit disturbances, regardless of their causes. However, the analysis discussed above demonstrates important differences in the erythropoietin and red blood cell production responses for these two cases. In order to achieve closer agreement with the animal data and produce the results shown here, it was necessary to include the additional factors addressed in Figure 32 in simulating the dehydration case. These factors included alterations in blood flow (a decrease of 10 percent during the first day which, by an assumed renal autoregulatory effect, returns to normal thereafter), reduction in maturation time to 25 percent of that used in the transfusion case, and a decreased responsiveness of hemopoietic tissue as dictated by experimental results (Figure 30).

With the addition of these hypotheses, the model demonstrated the capability of simulating different responses for dehydration and transfusions and the model responses compare favorably with those found experimentally. Physiologically, the two most important aspects of this simulation are a delay in erythropoietin suppression during dehydration, but not after transfusion, and a delay in suppression of red cell production after transfusion, but not during dehydration. Thus, the dissociation between erythropoietin and red cell production during dehydration, explained by direct action of diet restriction on hemopoietic tissue which bypasses the erythropoietin pathway, coupled with a transient decrease in blood flow, was successfully reproduced in the simulation.

More detailed simulations were also performed of the dehydration experiments. Figure 35 shows the response for the mouse and for the mathematical model for 3 days of dehydration and 7 subsequent days of rehydration. The suppression of erythropoietin and red cell production is similar to that shown in Figure 34. During dehydration, the following parameters were adjusted in a direction in accord with the hypotheses presented in Figure 32 and, where possible, in a magnitude dictated by the experimental results: a) a decrease in plasma volume as shown; b) a transient

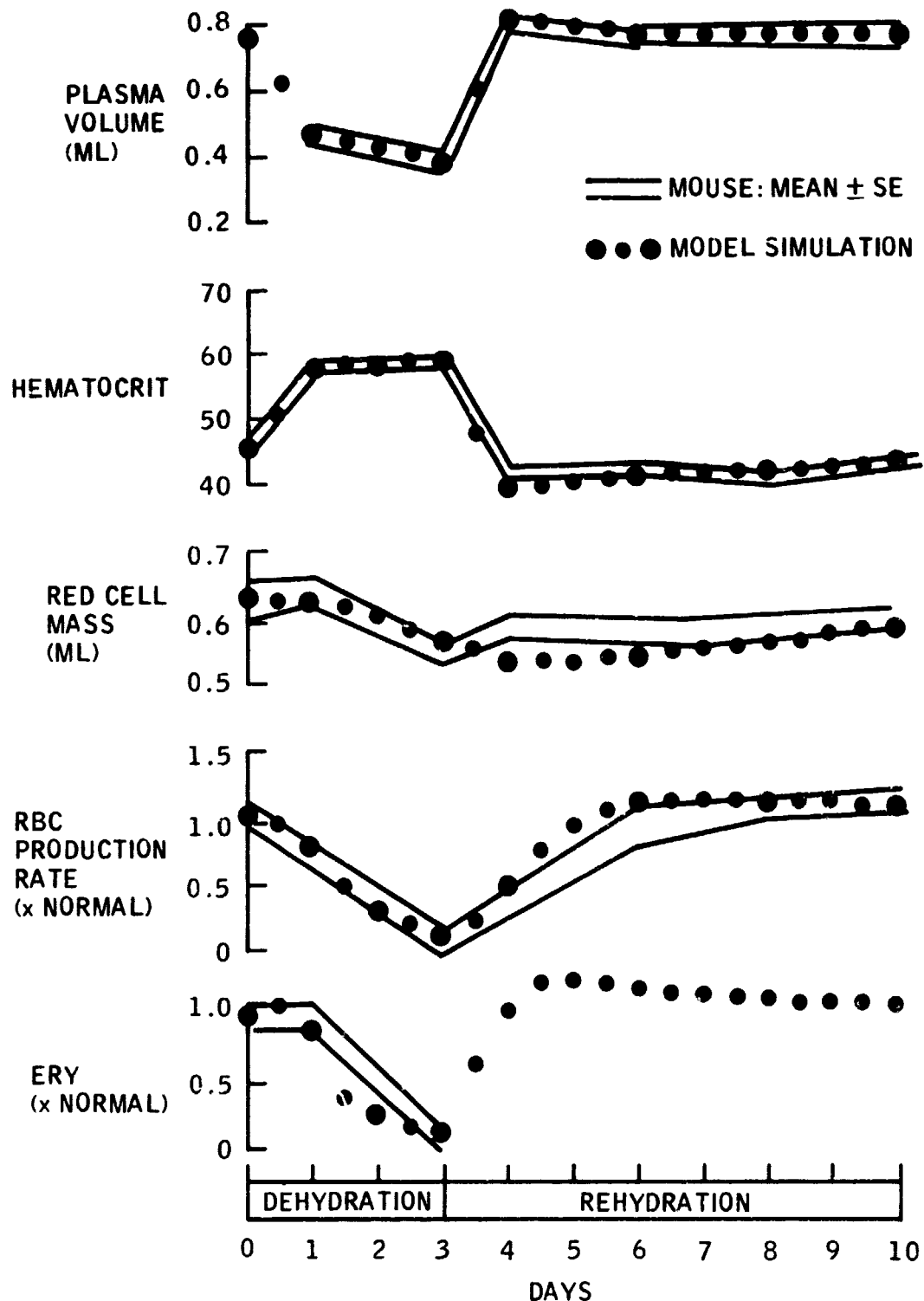


Figure 35: Simulation and experimental murine hematological responses to 3 days of dehydration and 7 subsequent days of rehydration. The shaded area indicates the standard error range of the mouse data, taken from a composite of several different experiments of Dunn and co-workers (1978, 1979a, 1980). The solid circles illustrate the model's responses. The larger solid circles represent the discrete points in time at which the animal data was taken.

decrease in blood flow (-10 percent) for the first 24 hours; c) a gradual decrease in responsiveness of hemopoietic tissue to erythropoietin to 20 percent of control; d) a gradual decrease in oxygen uptake to 80 percent of control; and e) a reduction of the time (i.e., maturation time) required for erythrocyte production to respond to influencing factors. During rehydration, these parameters were returned to normal and only the plasma volume influence was used to drive the simulation. This choice of parameter adjustments were established by a trial-and-error procedure, continually testing the model response against the experimental data.

Once a reasonable simulation has been achieved, as demonstrated in Figure 35, it is often instructive to determine the relative influence of each major factor on the final response. This is accomplished easily with a model by first performing the optimal simulation with all the required hypotheses and then selectively removing one or more of the effects (i.e., setting the model parameter at its control value) and performing the simulation again. In this particular case, it is convenient to think of the dehydration response as being under the influence of a negative water balance effect and a negative energy balance effect. Of the five hypotheses described in the paragraph above, the first two (plasma volume and blood flow effects) are related to water balance and the remaining hypotheses (hemopoietic responsiveness, oxygen uptake, and transit time) are related to energy balance effects. Two simulations were performed, as shown in Figure 36, to examine the separate influences of these water balance and energy balance factors on the red cell mass, red cell production rate, and erythropoietin levels.

It is clear that the effect of the hypotheses related to a negative energy balance has a more profound influence on erythro-suppression than the hypotheses related to a negative water balance. However, it should be noted that the suppression of erythrocyte production during the water balance simulation, while less acute, is quite similar in magnitude to that predicted for bed rest (e.g., see Figure 26). And, while in bed rest a negative water balance is a sufficient stimulus to decrease the red cell mass significantly given sufficient time, the three-day rehydration experiment is far too short to produce a noticeable effect on red cell mass in this simulation. Most of the decrease in red cell mass during short-term rehydration is, therefore, attributed to the dramatic inhibition of red cell production as induced by

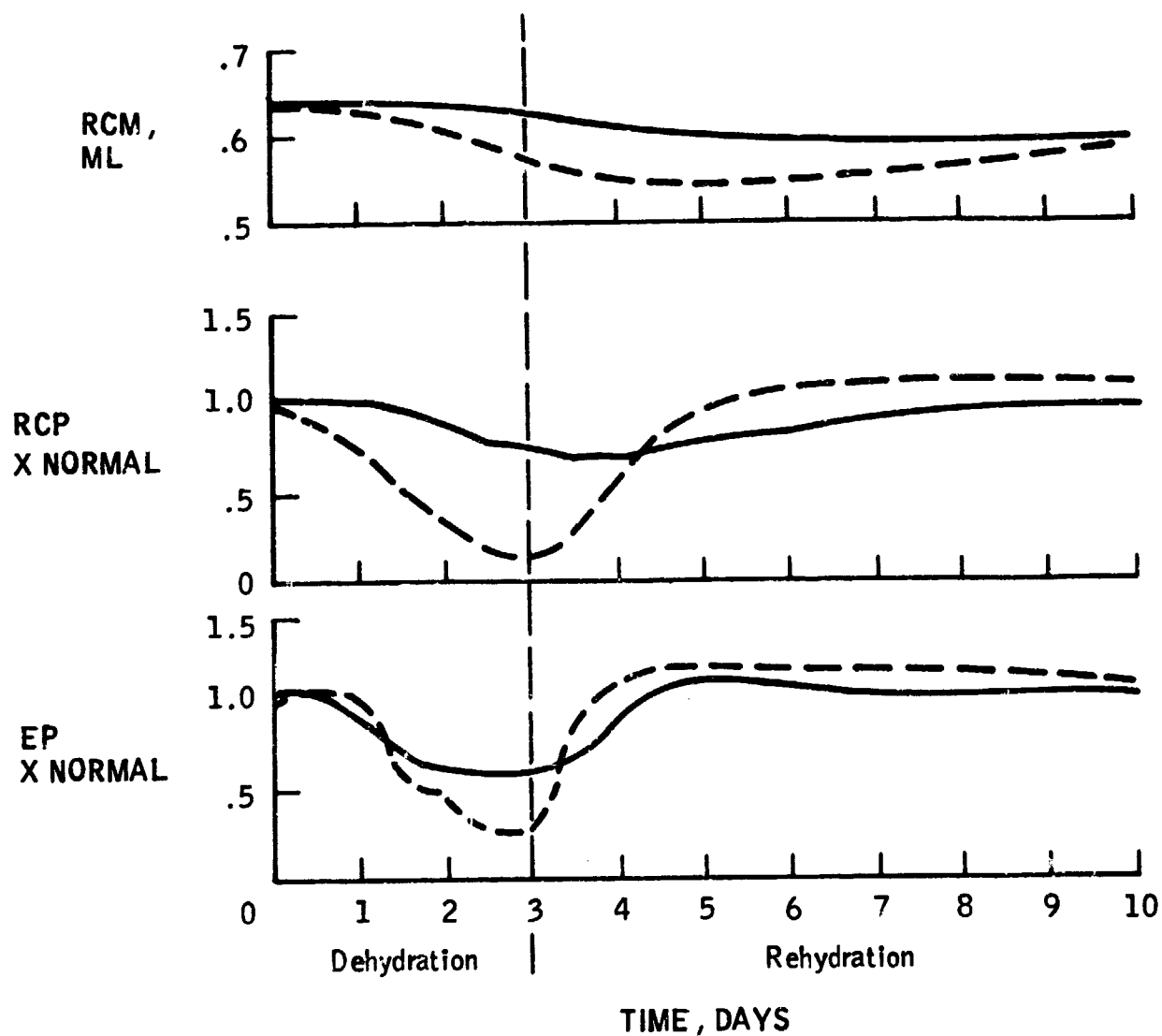


Figure 36: Simulated responses of "mouse" model showing the separate effects of water balance factors (solid line) and energy balance factors (dashed line) in producing the combined response illustrated in the previous figure. See text for description of parameter adjustments during each simulation. (RCM = red cell mass, RCP = red cell production, EP = erythropoietin).

energy balance factors. Also of interest is the fact that erythropoietin levels for the two cases considered are similar, when compared to their quite dissimilar effects on red cell production and red cell mass. The entire erythropoietin effect in the energy balance simulation is due to the reduction in oxygen uptake.

The computer simulations were particularly valuable in revealing unusual trends in the data and suggesting clarifying experiments. A summary of the major problem areas that were resolved with the assistance of the model are listed in Table 12. The first column represents the trends in the data which were not easily explained by the original computer analysis or by intuition. One or more hypotheses which would resolve the discrepancy as suggested by the simulation approach are shown in the second column. The last column indicates the results of second generation experiments which were performed to test the hypotheses in the laboratory. The model behavior suggested that all of the hypotheses listed in Table 12 were theoretically credible, but, as shown, the experimental evaluation proved that some of them were not biologically plausible. The interaction between modeling and animal studies demonstrated that the hemoconcentration effects (i.e., plasma volume changes) and negative energy balance effects (i.e., reductions in tissue sensitivity to erythropoietin and metabolic rate) were the most influential in accounting for the experimental findings (items 1, 2, and 6 in Table 12). However, in order to bring all of the simulated variables into agreement with the experimental findings, several additional factors needed to be considered, which eventually produced the simulations presented in Figure 35. These factors, shown in Table 12 (items 3, 4, and 5) are discussed in the next three paragraphs.

The red cell mass decreased more rapidly in vivo than was initially predicted by the computer. The animal data could be simulated only by assuming a 50 percent increase in the rate of red cell hemolysis during dehydration. However, estimation of serum bilirubin levels failed to support this concept. Survival of ^{51}Cr -labelled red cells also failed to support the concept of increased hemolysis during dehydration. Nevertheless, these measurements demonstrated that the normal red cell lifespan in the particular strain of mice used to study dehydration was about half the value used initially in the computer model (which was based on accepted literature data;

TABLE 12

DEHYDRATED MOUSE STUDY:
HYPOTHESES FORMULATED AND TESTED TO EXPLAIN DISCREPANCY BETWEEN
EXPERIMENTAL AND SIMULATION RESPONSES

PROBLEM	HYPOTHESIS REVEALED BY SIMULATION	EXPERIMENTAL EVALUATION
1. Explain reduction in red cell production during dehydration	Erythropoietin suppression induced by tissue hyperoxia secondary to hemoconcentration	Hemoconcentration confirmed; tissue oxygenation not measured; EP not suppressed on first day
2. Explain reduction of erythrocyte production despite normal EP titers on first day of dehydration	Reduction in sensitivity of hemopoietic tissue to EP	Confirmed for spleen, but not for bone marrow
3. Explain normal EP titers on first day despite hemoconcentration and reduced erythrocyte production rates	a) Reduced sensitivity of renal tissue to tissue oxygenation b) Increased EP half-life c) Increased oxy-hemoglobin affinity d) Reduced blood flow	a) Ruled out b) Ruled out c) Ruled out d) Not measured; cannot be ruled out
4. Explain larger decrement in red cell mass during dehydration than is predicted by model	Reduction in red cell life span	Hemolysis ruled out, but normal lifespan found to be shorter than originally believed
5. Explain rapid fall in erythrocyte production on first day of dehydration despite maturation time delay	Reduced response time of hemopoietic tissue to EP (i.e., shorter time delay)	Confirmed for splenic tissue
6. Explain more rapid and extreme fall in EP titers after first day of dehydration than is predicted by model on basis of hemoconcentration	Delayed reduction in oxygen uptake	Confirmed

e.g., Abbrecht and Littell, 1972). Adjustment of the computer parameter for red cell destruction to agree with more new experimental findings (5.3 percent per day, corresponding to a lifespan of 20 days rather than the original value of 40 days) resulted in a good simulation of the experimentally-determined changes in red cell mass, not only during dehydration, but also during rehydration. Thus, the model's prediction that red cell life span should be shortened was verified in the laboratory.

Even when appropriate changes in metabolic rate and tissue sensitivity to erythropoietin were incorporated into the computer simulation, the rate of red cell production did not fall as rapidly as was determined experimentally. Therefore, the computer simulation shown was obtained by reducing the transit for red cell precursors through hematopoietic tissue to one day from its nominal value of 3 to 5 days (Gurney et al., 1966; Kirk et al., 1968). Experimental justification for this maneuver is based on the following evidence: spleen and, particularly bone marrow cellularities show marked decreases within 24 hours of commencing dehydration (see Figure 30), and proliferation of normal marrow stem cells in vitro is more rapid for the strains of mice used in these experiments than in other strains, suggesting a normally shorter transit delay in red cell production (CDR Dunn, unpublished observation).

In the computer simulations, an increase in hematocrit, whether induced by red cell transfusion or a reduction in plasma volume, resulted in an immediate decrease in serum titers of erythropoietin (i.e., see Figure 25 and 26). In contrast, in the animal studies erythropoietin remained normal for at least the first 24 hours of dehydration. At least three factors were considered to bring the computer simulations into agreement with the experimental findings: a) a change in the sensitivity of the erythropoietin producing system; if the hormone producing system became less sensitive to increases in tissue oxygen then, theoretically, this might be expected to maintain erythropoietin titers near normal; b) a change in erythropoietin half-life ($t_{1/2}$); if erythropoietin $t_{1/2}$ is related to marrow erythroid activity (Stohlman, 1962) then suppressed erythropoiesis might be expected to extend $t_{1/2}$ which, in turn, would act to maintain serum erythropoietin titers near normal; c) a shift in blood P_{50} ; a decrease in P_{50} would diminish oxy-hemoglobin affinity and the degree of oxygen unloading at the tissue level, thus tending to increase erythropoietin

levels; d) a change in renal blood flow; theoretically, the reduced plasma volume would be expected to increase blood viscosity and passively permit blood vessels to shrink in diameter. Both of these effects would reduce blood flow and oxygen transport. The first three assumptions (a, b and c) were examined experimentally (Dunn et al., 1981) and found not to change. However, other investigators have shown a reduction in the sensitivity of erythropoietin release to hypoxia following dehydration (Giglio et al., 1979). As far as blood is concerned, it was assumed that a reduction in renal blood flow could occur only during the first day in light of the excellent flow autoregulatory capability of the kidney. This remains a hypothetical but perhaps logical explanation for the maintenance of normal erythropoietin titers, despite an increased hematocrit.

These studies illustrate the potential usefulness of the interaction of experimental and computer studies in the systematic analysis of alterations in the control systems of specific biological processes. Thus, the red cell lifespan, oxygen uptake, sensitivity of the erythropoietin producing system, and erythropoietin half-life and blood P_{50} would probably not have been measured if experimental and computer studies had been originally in agreement. Also, certain concepts such as the effects of total blood volume and blood flow on tissue oxygenation, and of red cell marrow transit times on erythropoietic dynamics would probably not have been considered to explain the data obtained in the dehydrated mouse if computer simulations had not been undertaken.

In summary, these animal and computer studies have shown that hemoconcentration is a major factor in producing erythro-suppression in both absolute and relative erythrocytosis. However, when hemoconcentration is caused by water-restriction, an additional factor is present that reduces the responsiveness of hemopoietic tissue to erythropoietin. The negative energy balance that accompanies dehydration in mice was implicated as a causative agent in this mechanism. The hypovolemia of dehydration may have contributed to a transient decrease in blood flow, which together with the reduced insensitivity of hemopoietic tissue accounts for a temporary dissociation between erythropoietic activity and its hormone regulator. Extrapolating to the space-flight situation, it is suggested that the negative energy balance may contribute to the loss in red cell mass to a degree that had heretofore

not been appreciated. Food intakes which are lower than necessary to maintain energy balance and body mass have often been observed in astronauts (Leonard, 1982).

From the point of view that dehydrated mice and space travelers have both been observed to experience a negative water and negative energy balance which leads to hemoconcentration and reduction in erythropoietic activity, the dehydrated mouse may be considered to be a suitable ground-based analog of the hematological response to weightlessness. However, there are several important differences between the humans exposed to zero-g and their dehydrated animal counterparts: a) hemoconcentration of astronauts may not necessarily occur by fluid restriction, but rather by excess renal excretion secondary to headward fluid shifts; b) the reduced body fluid volume of space travelers is believed appropriate for health in zero-g while dehydrated mice cannot be maintained for more than several days because of water and nutritional deficiency; c) body fluid composition of astronauts are more likely to be normal compared to dehydrated mice; and d) food restriction in human space travelers have typically been observed in conjunction with space sickness, which is not always present, while food restriction in mice always accompanies fluid restriction. Therefore, these animals can, at best, represent the human condition in zero-g for only a brief period of time and only when both food and water restriction is evident.

Space-flight Simulations

Many factors have been discussed which could have contributed to the anemia of space flight. The available inflight data, as sparse as it is, together with the analysis of human and ground-based studies, does permit speculation to center on several probable pathways. It seems plausible to suggest that the enhanced oxygen carrying capability of hemoconcentrated blood and the decreased energy balance of a restricted diet may have limited the production of erythrocytes. Other factors which cannot presently be ruled out, but which are believed to be capable of exerting a secondary influence, include the possibility of some red cell destruction, the influence of a slightly hyperoxic environment, shifts in oxygen-hemoglobin affinity, changes in total blood volume, blood flow alterations, decrements in lean body mass, and the varying levels of exercise performed by the space crews.

The problem which remains is to relate these factors, not only to the absolute fall in red cell mass, but to the varying degrees to which red cells disappeared in the different Skylab missions as well as to the non-uniform kinetics of postflight recovery. Within limits, the techniques of computer simulation have proved valuable in integrating the available data with current concepts of erythropoietic regulation and choosing between alternative zero-g hypotheses. These simulation studies will be presented in this section in three broad areas: suppression of red cell mass during flight; postflight recovery of red cell mass; and comparison between bed-rest and space-flight erythrokinetics.

Suppression of Red Cell Mass During Space Flight:

The kinetics of red cell mass disappearance during flight is unknown. Two theories have been advanced to explain inflight erythrokinetics and to account for the different net red cell mass losses observed on the three flights (see "Differences Between Missions"). Evaluation of these two divergent concepts, termed the "regeneration theory" and the "continuous loss theory" will be described below.

i) Evaluation of Regeneration Theory

The regeneration theory hypothesizes that red cell mass decrements occur within the first month of flight and that repletion of cells begins shortly thereafter regardless of whether the human subjects return from space. In the previous discussion of this theory, it was argued that while regeneration is theoretically a possibility, the supporting data are inconclusive. In particular, the suggestion by others (Kimzey, 1975, 1977) that a temporary hemodilution (observed during a single inflight day) could trigger the regeneration of red cells over the next month was deemed unlikely. The improbability of this event received support, inadvertently, from a bed-rest study in which a large amount of ingested saline was tested as a counter-measure for orthostatic intolerance (see Figure 6). The resulting temporary hemodilution did not appear to alter the course of red cell disappearance. This conclusion was supported by computer simulation studies, although a small effect on erythrokinetics of the bone marrow was demonstrated (see Figure 26).

There are factors other than inflight hemodilution which can be conceived to be responsible for regeneration of red cell mass. Simulation analysis has suggested several guidelines which can be used in formulating hypotheses: regeneration is only possible if production rates of red cells increase above destruction rates; a biphasic regeneration response (i.e., Figure 7) is not likely to occur when two competing stimuli, one erythro-suppressive and the other erythro-active, are initiated simultaneously, and are maintained for the same length of time; and a biphasic red cell mass recovery curve becomes more credible by assuming that an early erythro-suppressive influence is followed by an erythro-active stimuli.

It can be assumed that one suppressive influence on red cell production is that of hemoconcentration, which appears to persist at progressively diminished levels throughout three months of space flight (Figure 23). However, experimental and simulation studies have shown that a chronically maintained relative polycythemic condition will not, by itself, allow production rates to rise to levels which promote red cell mass regeneration (See Figure 26 and 28). Therefore, it is likely that additional factors or events must be postulated to account for regeneration. Two scenarios have been postulated: a) a stressor acting early inflight reduces the red cell mass within the first few days or weeks and thereafter its influence is greatly diminished or entirely disappears, thereby permitting normal or enhanced erythropoiesis. Examples of such a stressor which would be compatible with space-flight data may include hemolytic destruction of cells early inflight or restricted dietary intake early inflight; b) some stimuli not previously present increases production rates late inflight. Such an effect could be induced by some as yet unknown adaptive influence of zero-g on oxygen transport (i.e., shifts in oxy-hemoglobin affinity, renal blood flow) or the bone marrow. However, there is currently no evidence of any kind for such a mechanism during space flight or bed rest, and this possibility will not be considered further.

With regard to the first scenario of an early acting stressor, two hypotheses were examined by simulating the 59-day Skylab mission. In the study illustrated in Figure 37, it was assumed that the renal-bone marrow controller exhibited a reduction in responsiveness (i.e., change in sensitivity and threshold) during the first month in space. The suppression

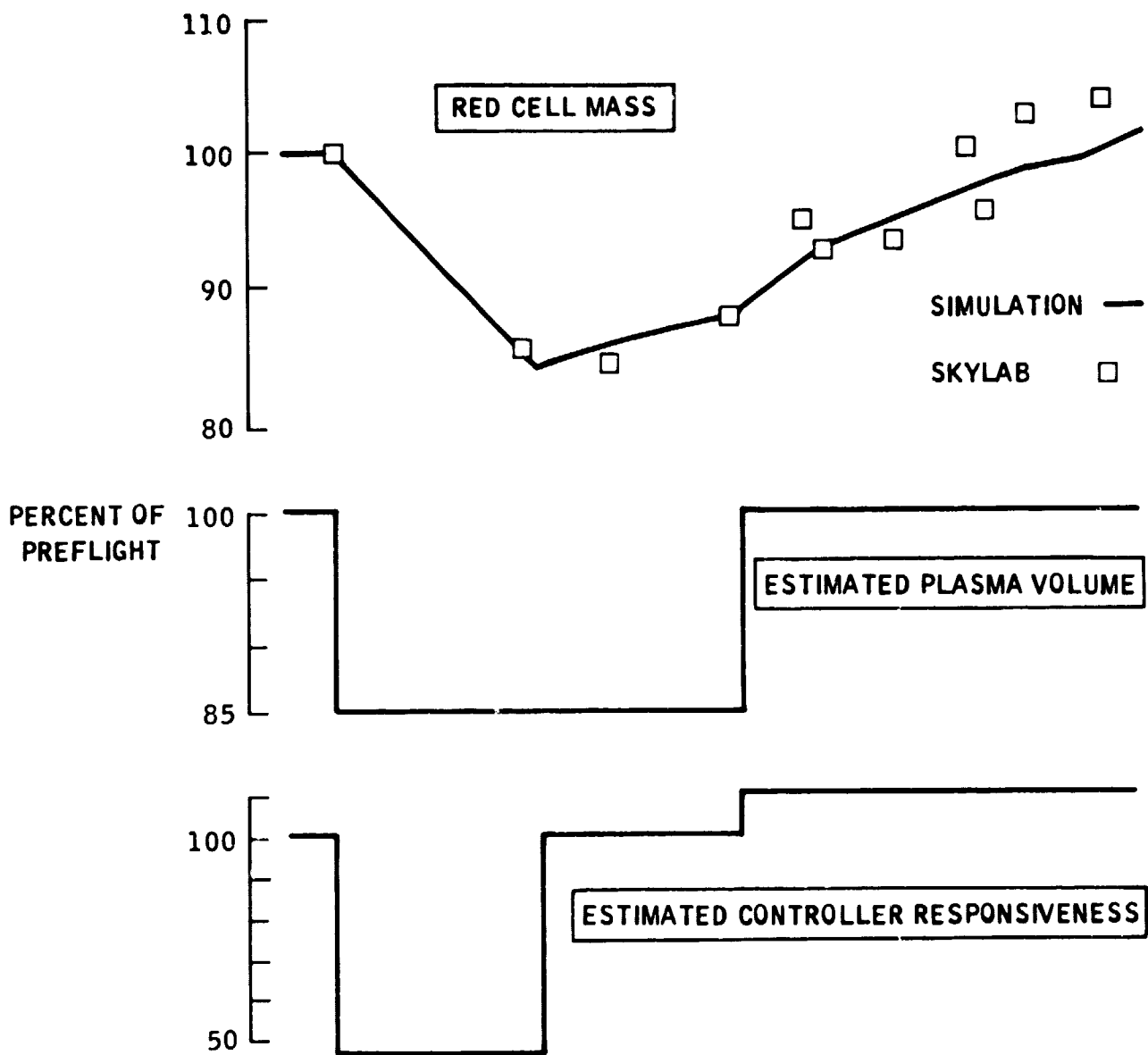


Figure 37: Simulation of the 59-day Skylab mission illustrating the hypothetical conditions under which regeneration of red cell mass may take place. Plasma volume was assumed to decrease during the entire inflight phase and controller responsiveness was assumed to decrease only during the first month. This resulted in the decline of red cell mass. Regeneration took place following hemodilution (return of plasma volume to normal) and a postulated increase in controller responsiveness. The experimental red cell mass behavior is a composite of postflight measurements obtained from the nine Skylab crewmen (Kimzey et al., 1976b).

was removed during the second month and a small enhancement in responsiveness was assumed thereafter. A concomitant reduction in plasma volume was imposed for the first two months before returning to normal as suggested by hematocrit changes and bed-rest studies. The rationale behind the change in controller sensitivity was not well formulated at the time this simulation was first performed (Kimzey et al., 1976b). Subsequently, the work with dehydrated mice suggested that such a parameter alteration is a somewhat plausible representation of the influence of the negative energy balance observed in the 59-day Skylab mission (Rambaut et al., 1977). In that flight, the severe symptoms of space motion sickness impaired normal dietary intake for the first mission week. Thus, an early reduction in red cell mass could have occurred on two accounts; hemoconcentration, and reduced dietary intake. Later inflight, the dietary intake increases toward normal while the hemoconcentration effect diminishes somewhat, as red cell mass is lost. This might favor increases in bone marrow production and a partial restoration of red cell mass.

While this simulation of the 59-day mission agrees with the regeneration curve composed of data from all three flight crews, corresponding simulations of the 28-day and 84-day missions did not show similar agreement for two reasons. First, dietary restriction was not severe on the shortest flight and almost non-existent on the longest flight, so that the controller parameters would not be expected to change to the same extent as shown in Figure 37. Secondly, plasma volume levels should return one month sooner than shown for the 28-day flight and one month later than shown for the 84-day flight. When these changes were instituted, the inflight red cell mass response curve departed widely from the regeneration curve based on postflight crew data.

A second hypothesis to explain regeneration was examined in which it was assumed that a hemolytic loss of red cells took place during the first several days of the mission. Such a loss, if it occurred selectively in older cells, would not necessarily be measured by lifespan techniques (see "Red Cell Destruction"). Figure 38 illustrates the simulated responses in two cases: a short period of hemolysis (10 percent of cells destroyed), and hemoconcentration produced by a sustained reduction in plasma volume. The latter case is similar to previous illustrations of hemoconcentration (e.g., Figure 28). Hemolysis (solid line) results in a biphasic regeneration response of red cell mass, but since hematocrit and erythrocyte production rate responses are opposite to expected or measured values, it is not likely that hemolysis by

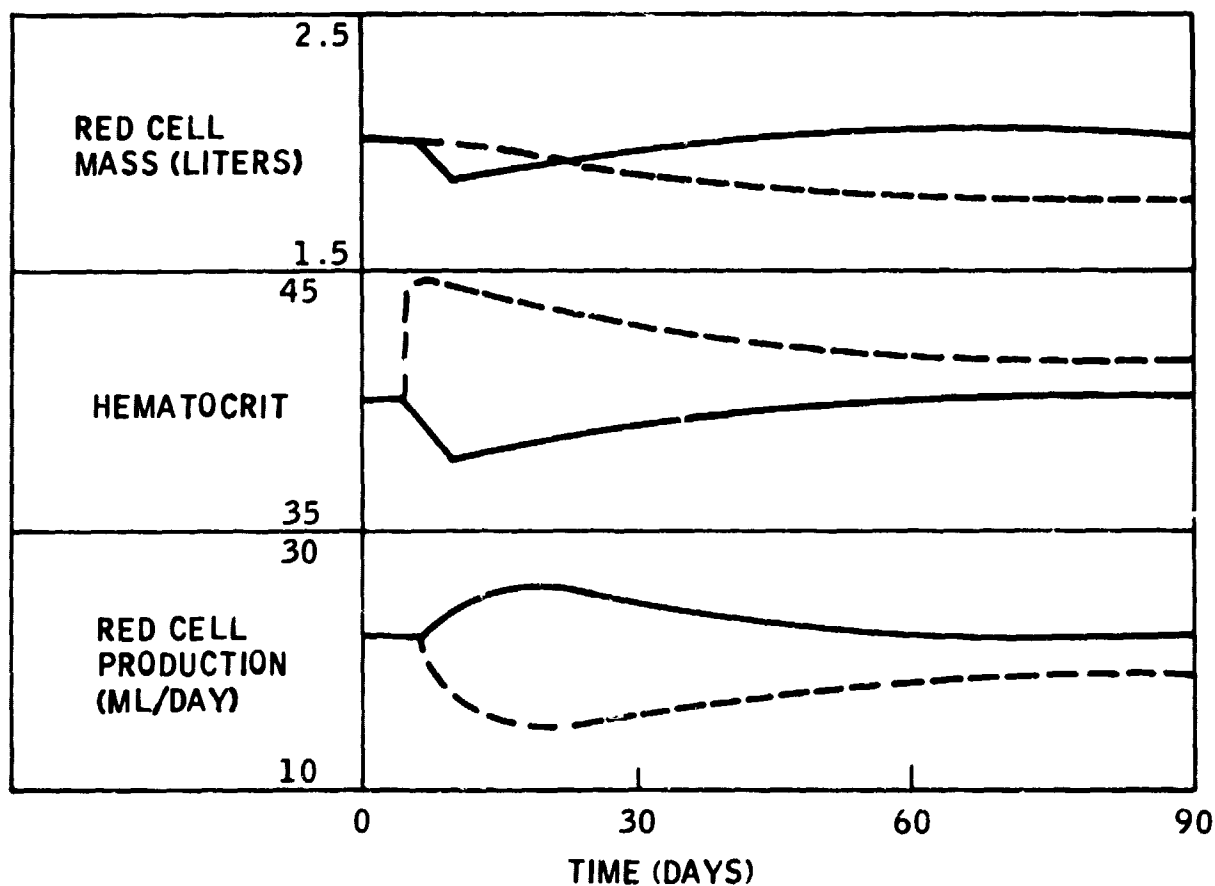


Figure 38: Simulation analysis comparing loss of red cell mass by either hemolysis of 10 percent of the circulating red cells during the first 3 days (solid line) or plasma volume reduction (-500ml) maintained throughout the period studies (dashed line). Acute hemolysis produces a biphasic regeneration response, but the hematocrit and red cell production are opposite to the responses expected for hemoconcentration alone. The effect of both stresses, hemolysis plus hemoconcentration, acting together are shown in the next figure.

itself can account for either the entire space-flight loss of red cell mass or its regeneration. Hemoconcentration alone (dashed line) does account for loss in red cell mass, but as suggested earlier, a regeneration response is not possible by this mechanism.

The simultaneous occurrence of hemoconcentration and hemolysis is illustrated in Figure 39. Two responses are shown, the solid line representing the case where hemolysis destroys 10 percent of the cells, and the dashed line representing 20 percent of cell destruction. A plasma volume reduction of 500 ml was imposed on both simulations. If cell destruction is large (dashed line), a biphasic regeneration curve of red cell mass is apparent, as in the case of hemolysis by itself. However, the accompanying hematocrit response is triphasic, quite unlike the observed inflight data. On the other hand, for smaller degrees of hemolysis that are not large enough to reduce hematocrit to below control levels (solid line), hemoconcentration is evident throughout the response period and production rates are below control as expected. Also, in this case, the red cell mass no longer exhibits regeneration characteristics. In fact, the simulation predicts that the long-term reduction in red cell mass is the same whether or not hemolysis occurs; it is only the short-term response which results in enhanced red cell mass loss due to the addition of hemolysis.

It becomes apparent that alterations in hemoglobin levels consistent with those observed during Skylab could not by themselves generate computer simulation responses comparable to the observed changes in red cell mass, if one accepts a composite view of the postflight data. These analyses suggest that some degree of regeneration of red cell mass may have occurred in isolated instances such as during the 59-day mission when severe space motion sickness resulted in large temporary decrements in food intake. But it is difficult to demonstrate, on the basis of plausible hypotheses, that regeneration is a universal phenomenon of space travelers as originally suggested (Kimzey, 1977). The simulation and experimental studies have tended to rule out inflight hemodilution, negative energy balance, or hemolysis as causative factors in the regeneration phenomenon. However, it has been impossible to rule out a modest early inflight hemolysis event as a contributory factor to red cell mass loss (without regeneration) during space flight.

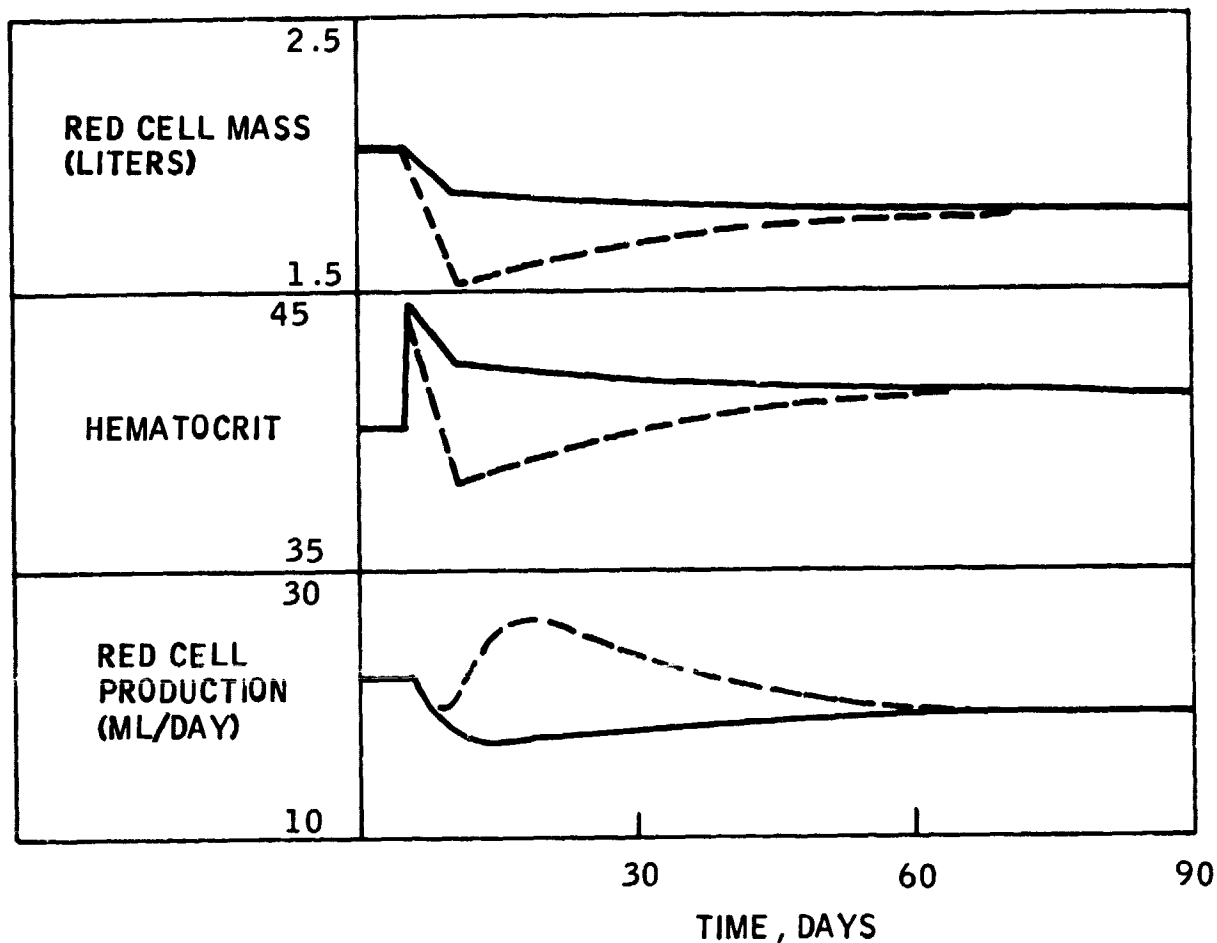


Figure 39: Simulated analysis of hemolysis as a possible causative factor of regeneration. In both cases shown, the plasma volume was reduced stepwise by 500 ml and this was combined with an acute 3-day hemolysis simulated by transiently reducing the red cell lifespan. Qualitatively different responses are seen when either 10 percent (solid line) or 20 percent (dashed line) of the circulating red cell mass is destroyed by hemolysis.

ii) Evaluation of Continuous Loss Theory

The continuous loss theory specifies that red cell mass depletes gradually as a function of time in weightlessness, similar to the behavior seen in bed rest. Furthermore, the differences in red cell mass loss among the Skylab crewmembers who remained in space for varying time periods is assumed not to result from any adaptive or regenerative process. Rather, it is proposed that factors which influence erythropoietic activity are present in different degrees in the three missions. The net result of these factors is presumed to be a diminished erythropoiesis which is greatest during the shortest Skylab flight and least during the longest flight.

The most promising candidates which have been identified as erythro-suppressive factors and which were known to be present during space flight are those related to hemoconcentration and negative energy balance. Energy balance is determined by diet and physical activity, and it is assumed that both of these were adequate on the longest flight and grossly inadequate on the shortest flight (Thornton, 1978). It is known that dietary restriction suppresses erythrocyte production, and it will be assumed that a reduced amount of activity has the same effect (see "Effect of Diet" and "Effect of Exercise"). These three factors, hemoconcentration, diet, and activity, and an estimate of their varying presence on the Skylab missions are given in Table 13. Two other factors are also listed, hyperoxic blood and hemolysis, which could have been present on the 28-day mission (see Table 3 and Table 9) to a modest extent. The analysis of Table 13 provides a rational basis for qualitatively predicting that the greatest red cell mass loss occurred on the shortest mission and the smallest loss occurred on the longest mission. These factors were subjected to further quantitative examination using simulation and statistical analysis.

Evaluation of the continuous loss theory was accomplished in two stages. First, the influence of hemoconcentration (negative water balance) on red cell mass was ascertained by computer simulation. Secondly, the differences in energy balance factors were described and tested.

The hematological responses of the Skylab crew which resulted only from the influence of hematocrit alterations were estimated by computer simulation. Figure 40 and 41 illustrate the computer-generated responses for the 59-day and 84-day missions, respectively, using only the mean inflight plasma hemoglobin concentration data to drive the model. (The 28-day mission was not

TABLE 13

RELATIVE IMPORTANCE OF FACTORS PROPOSED TO BE RESPONSIBLE
FOR REDUCED RED CELL MASS ON SKYLAB*

<u>Factor</u>	<u>28-Day Mission</u>	<u>59-Day Mission</u>	<u>84-Day Mission</u>
Hemoconcentration	- **	-	-
Dietary Intake	=	-	0
Physical Activity	=	-	0
Hemolysis	-	0	0
Arterial PO ₂	-	0	0
Red Cell Mass	=	=	-

* The number of dashes indicates the relative contribution of each factor toward the decreased red cell mass based on that factor's relative intensity on the three flights. Factors are ranked horizontally only; ranking in the vertical direction has no meaning. "0" indicates no effect of factor on RCM; "=" indicates maximum Skylab effect.

** Inflight Hb concentration was not measured on the 28-day mission, but it is assumed that hemoconcentration was similar to the other flights. Accordingly, this table reflects the hypothesis that hemoconcentration caused a similar degree of erythro-suppression on all three missions.

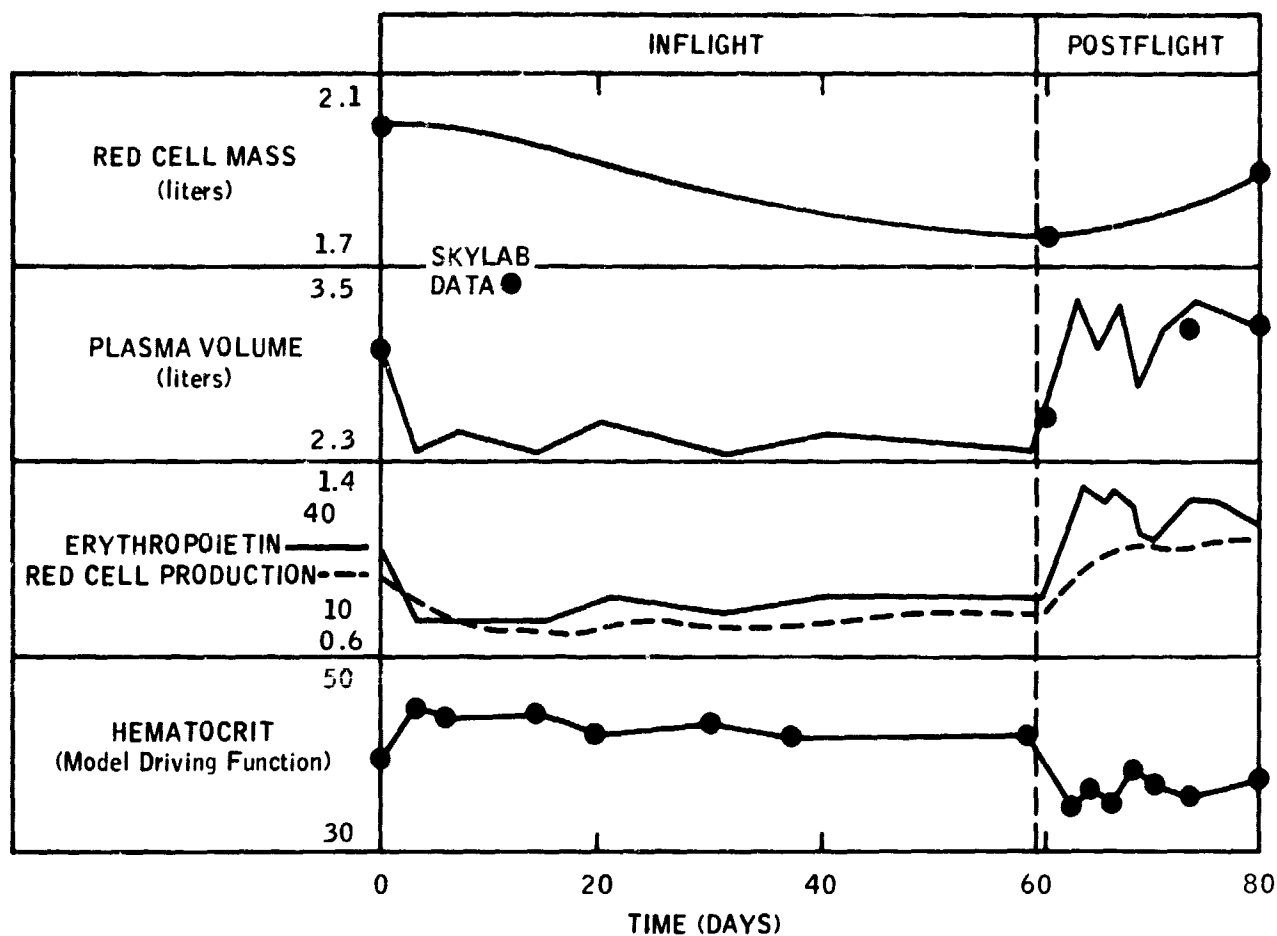


Figure 40: Simulated hematological response of the crew of the 59-day mission. The response of the erythropoietic model was generated by using the measured hematocrit-time profile as the only driving function. In addition to a gradual loss in red cell mass, the model predicts more rapid decreases in plasma volume, erythropoietin and red cell production. Red cell mass and plasma volume data obtained postflight compares favorably with the simulated response. Controller gain, G , was set to 5 throughout the simulation.

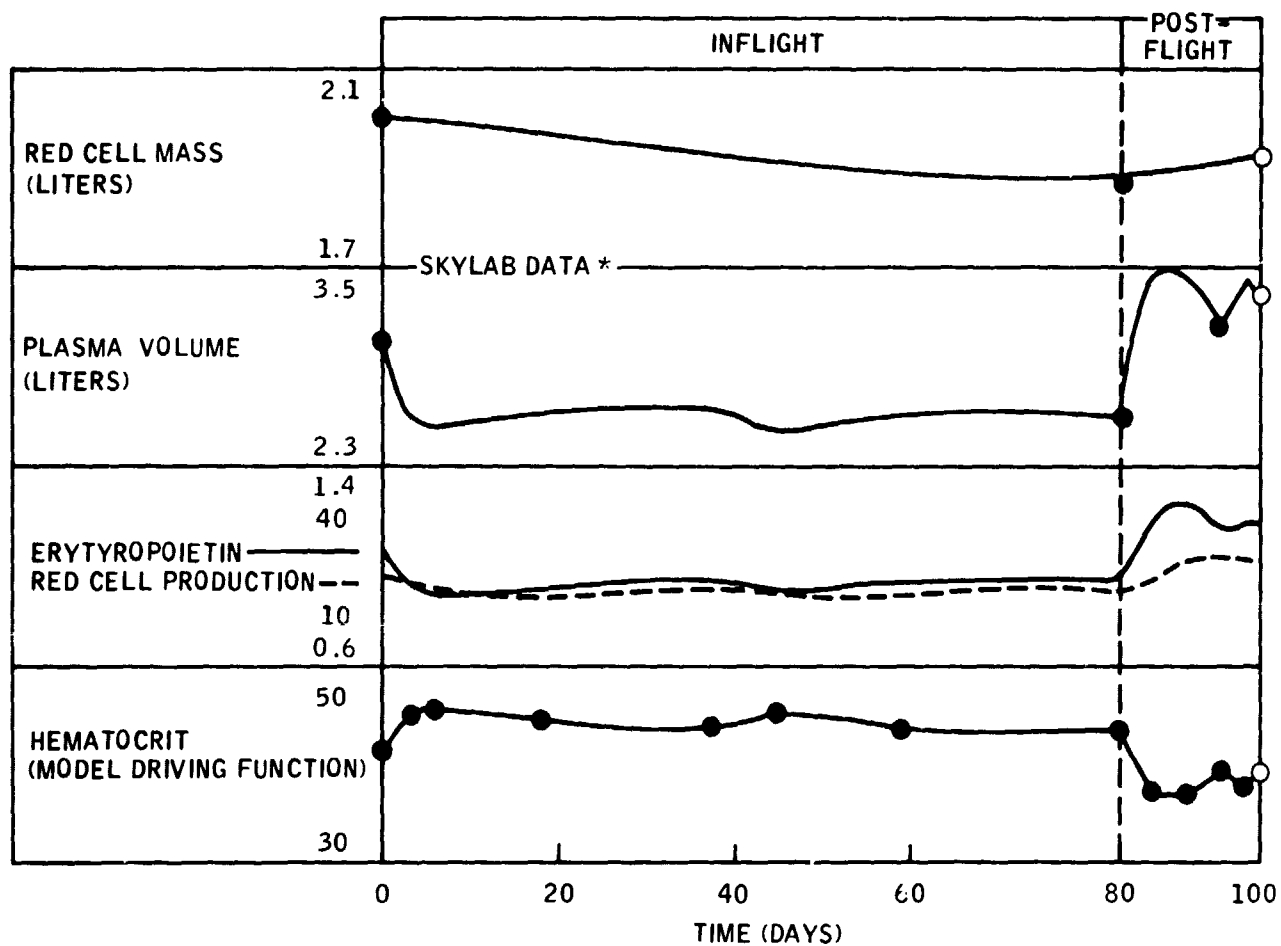


Figure 41: Simulated hematological response of the crew of the 84-day mission. See caption of previous figure. Controller gain, G , set to 2 throughout simulation.

simulated because no inflight Hb data were obtained on that flight.) This procedure is identical to that used in the bed-rest analysis (Figure 26). The postflight values of plasma volume and red cell mass (no inflight measurements were determined for these quantities) are indicated by solid circles. The gain factor, G, was adjusted (and held constant throughout the simulation) so that the simulated total inflight red cell mass loss agreed with the measured value obtained on recovery day. No further gain adjustments were made to simulate the postflight period. Agreement between model and data during the postflight period for red cell mass and plasma volume is considered good.

These simulations represent the first systematic attempt to predict, on a continuous basis, inflight red cell mass and quantities associated with its regulation using actual flight data. The regulatory basis by which decrements in erythropoietin, red cell production, red cell mass, and plasma volume are produced in the zero-g phase of the mission are quite similar to that described for the bed-rest study previously discussed. The inflight response is essentially a response to a relative polycythemia-induced-hyperoxia, and the postflight recovery response results from the dilutional anemia-induced-tissue hypoxia.

These simulations suggest, at first glance, that the loss of plasma volume during weightlessness can account for many of the changes in erythropoietic activity during flight and recovery. The similarity between these space-flight simulations and previously discussed bed-rest simulations, all in basic agreement with measured values, suggests a common etiology based on normal feedback regulation of erythropoiesis. It is encouraging that the two space-flight simulations exhibited similar trends and that both inflight and recovery phases could be estimated successfully using a single parameter adjustment for overall gain.

There was, however, one disturbing aspect of these simulations. The model, operating as a hemoglobinometer, predicts that the degree of erythro-suppression is proportional to the degree of hemoconcentration. It would, therefore, be expected that the similar levels of hemoglobin concentrations measured on the 59-day and 84-day missions (e.g., see Figure 23) would result in equal rates of loss of red cell mass on both flights and there would be greater losses at the end of 84 days in space than after 59 days in space. However, the measured red cell mass loss was almost twice as great for the 59-day mission than for the longest mission. This discrepancy

was empirically resolved during the simulations shown in Figure 40 and 41 by assuming that the sensitivity of red cell production to hemoglobin concentration (i.e., overall open loop gain, G) was two and one-half times higher for the longer flight compared to that of the 59-day flight. The gain factors required to obtain successful space-flight simulations was also quite different for those determined for other stresses examined, all of which were characterized by hemoconcentration (Table 14). The fact that the space-flight values differ by up to 600 percent compared to bed rest, infusions, or descent from high altitude, suggests that other factors in addition to hemoconcentration are present during space flight which have not been taken into account in the modeling analysis. (In simulation, it is often possible to use changes in gain to mimic some other effect which is not considered explicitly in the model.)

Other than hemoconcentration, energy balance factors appear to offer the most plausible explanation for red cell mass decrements. Using the results of a previous Skylab energy balance analysis (Rambaut et al., 1977; Leonard, 1980), it was found that the largest losses of red cell mass were associated with the crews who consumed the fewest calories ($r=0.71$, $p<0.05$), exercised the least ($r=0.63$, $p<0.1$) and lost the most tissue mass ($r=0.66$; $p<0.05$) (see Tables 15 and 16). A negative energy balance is a reflection of a decreased intake of calories and/or increased exercise expenditures, resulting in increasing amounts of endogenous tissue loss. On Skylab, it was believed that the increase in diet provided to the crewmen on the longer missions was greater than the accompanying increase in exercise, so that net energy balance became less negative or positive for those crewmen (Leonard, 1980). Taken as a whole, the correlations of Table 16 suggest that energy balance factors may indeed have been a factor in reducing red cell mass during space flight.

These statistical findings must be tempered, however, by the realization that the experimental design was not intended to reveal the caloric factors involved in erythropoiesis regulation. For example, the fact that diet and exercise both increased with increasing mission duration calls for caution in interpreting these results. (A regression of red cell mass loss vs. mission duration shows as strong a correlation ($r=0.75$, $p<0.05$) as that for red cell mass loss vs. diet).

TABLE 14

GAIN FACTORS USED IN SIMULATIONS WHEN HEMOCONCENTRATION
WAS THE ONLY STRESS CONSIDERED

<u>SIMULATION</u>	<u>GAIN VALUE REQUIRED FOR OPTIMAL FIT WITH DATA*</u>
Descent from Altitude	12
Red Cell Infusions	12
Bed Rest	10
Skylab: 59-day mission	5
Skylab: 84-day mission	2

* Gain refers to overall renal-bone marrow controller sensitivity relating tissue oxygenation to erythrocyte production rate (see Figure 12).

TABLE 15

SUMMARY OF INFLIGHT ENERGY BALANCE COMPONENTS
ON SKYLAB (N=3)

	<u>28-Day Mission</u>	<u>59-Day Mission</u>	<u>84-Day Mission</u>
Red Cell Mass Loss:			
(total ml)	311 ± 19	247 ± 123	134 ± 39
(ml/day)	11.1 ± 0.6	4.2 ± 2.1	1.6 ± 0.5
Diet (Kcal/d)	2930 ± 90	3225 ± 610	3260 ± 105
Exercise (W-m/d)	1950 ± 315	4690 ± 1620	4890 ± 715
Lean Body Mean Loss (gm/d)	- 60 ± 40	- 14 ± 26	- 10 ± 3
Fat Loss (gm/d)	- 41 ± 31	- 35 ± 24	+ 5 ± 11
Solid Tissue Loss (gm/d)	- 52 ± 34	- 40 ± 24	+0.7 ± 9

TABLE 16

ENERGY BALANCE FACTORS TESTED FOR THEIR INFLUENCE
ON RCM CHANGE (N=9)

<u>FACTOR</u>	<u>CORRELATION COEFFICIENT</u>
Diet ¹	- 0.71 ***
Bicycle Work ¹	- 0.63 **
Δ Lean Body Mass ²	0.61 **
Δ Body Fat ²	0.61 **
Δ Solid Body Tissue ²	0.66 ***

1. Total mission calories versus total mission red cell mass loss

2. Mass of tissue lost per day versus red cell mass loss per day

** $p < 0.10$

*** $p < 0.05$

Diet and exercise factors are not included implicitly in the computer model of erythropoiesis regulation. It was assumed that alteration of these quantities could be simulated by adjusting either of two model parameters, oxygen uptake (an exercise effect), and the operating position of the bone marrow controller function curve (possibly an exercise and dietary effect). Simulations of the three Skylab missions were accomplished using the influencing factors in Table 13 as a guide in the following manner: a) an inflight plasma Hb concentration vs. time function was constructed from the average of the two flights on which data were available, and this single function was used to drive each of the three mission simulations, (i.e., it was assumed that there was no difference in hemoconcentration among the missions); b) differences in dietary and metabolic activity were simulated by adjusting the controller operating point to values that provided a reasonable agreement between experimental and predicted red cell mass; c) a 10 percent decrease in red blood cell (RBC) lifespan was assumed for the 28-day flight in accord with the estimates presented in Table 3; d) a 7 percent increase in arterial oxygen tension was assumed for the 28-day flight in accord with the estimates presented in Table 9; and e) controller gain factors were held constant for all simulations ($G=10$) in accord with previous estimates for bed rest shown in Table 14. These parameter adjustments are listed in Table 17 (case 1) and the results of the simulations are shown in Figure 42, which includes the Hb drive function employed.

Additional simulations were performed, for comparative purposes, that used different parameter combinations and values. For simplicity, changes in arterial oxygen tension and red cell lifespan were ignored in these other cases, which are listed in Table 17. Instead, in cases 2 to 4, overall gain was chosen to be either 10 or 5, as shown, and optimal fits with the data were obtained by adjusting the operating point and oxygen uptake (case 4). A final simulation was performed (case 5) in which only the overall gain was adjusted. The simulated responses of these additional cases were essentially all similar to case 1, which is illustrated in Figure 42.

These simulations demonstrate the plausibility of the continuous loss theory. In order to account for the observed differences between Skylab

TABLE 17

ALTERNATIVE SCENARIOS WHICH PRODUCE NEARLY EQUIVALENT
SIMULATIONS OF THE THREE SKYLAB MISSIONS

	<u>28-Day Mission</u>	<u>59-Day Mission</u>	<u>84-Day Mission</u>
<u>Case 1</u>			
Overall Gain, G	10	10	10
* Operating Point, P1	+20%	+20%	+30%
Arterial Oxygen Tension	+ 7%	0%	0%
RBC Life Span	-10%	0%	0%
<u>Case 2</u>			
Overall Gain, G	10	10	10
* Operating Point, P1	-10%	+20%	+30%
<u>Case 3</u>			
Overall Gain, G	5	5	5
* Operating Point, P1	-20%	0%	+10%
<u>Case 4</u>			
Overall Gain, G	5	5	5
* Operating Point, P1	-2.5%	0%	+1.5%
* Oxygen Uptake	-2.5%	0%	+1.5%
<u>Case 5</u>			
* Overall Gain, G	14	5	2.5

Note 1: Percentages in table refer to changes with respect to normal model parameter value. A value of zero means that the normal value was used.

Note 2: The hematocrit versus time function that was used in these simulations is a composite of the 28-day and 59-day mission inflight data.

Note 3: (*) designates parameters which were adjusted to obtain optimal fit with data.

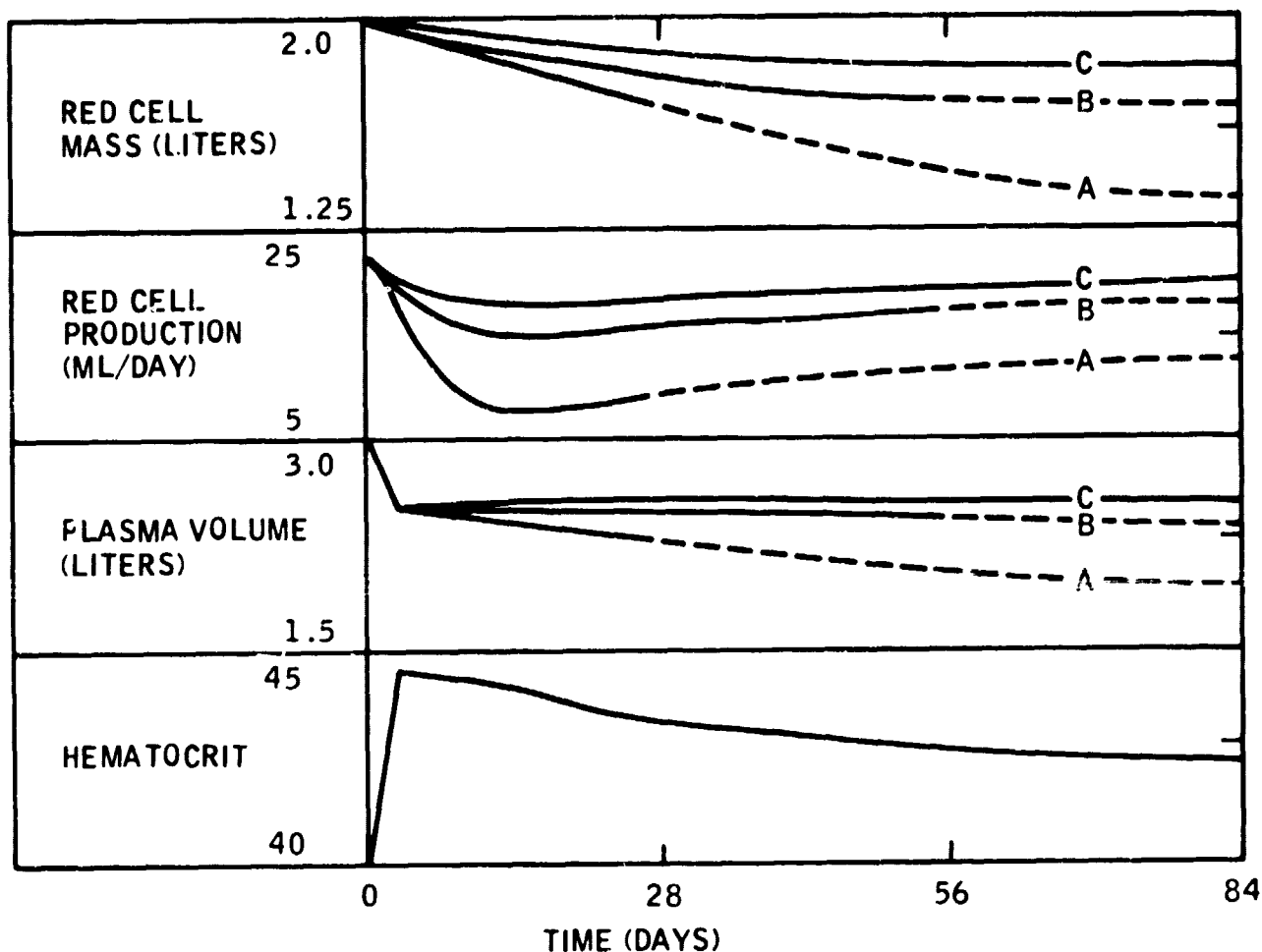


Figure 42: Simulation of the three Skylab missions: A = 28-day mission, B = 59-day mission, and C = 84-day mission. The hematocrit-time profile shown in the bottom panel was based on a composite of inflight data and was used to drive the model for the three simulations. In addition, the parameter adjustments shown in Case 1 were used. The dashed portion of the curves are predicted responses if all missions had continued for 84 days. The predicted end-of-mission values for red cell mass and red cell production are in good agreement with measured values. The predicted plasma volume losses are greater than measured at recovery for the two shorter missions. However, plasma volume measurements could be expected to be quite erratic at the end of space missions.

missions, it was not necessary to consider inflight regenerative behavior, nor a variable controller sensitivity (i.e., G was identical in all missions for cases 1 to 4), thus overcoming certain restrictions and discrepancies of earlier simulations (i.e., Table 14). Hemoconcentration can explain part of the loss of red cell mass, but it cannot explain the differences between missions. These differences may, however, be explained by other factors such as changes in controller function, oxygen uptake, arterial oxygen tension, and red cell lifespan. In each of the cases 2, 3, and 4, the values of P1 and oxygen uptake which provided good agreement between model response and data are in ascending order for the 28-day, 59-day, and 84-day missions, respectively, which would be expected if their values were somewhat proportional to average dietary and exercise levels of those crews. These simulations also indicate that more than one computer solution (i.e., combination of parameter values) can account for the observed losses in red cell mass. The predictive value of the simulation technique is thus limited by lack of inflight data. Nevertheless, the additional information that must be obtained in order to distinguish between the alternative scenarios is suggested directly by this study.

Postflight Recovery of Red Cell Mass:

An observation, selected for closer examination, was that after leaving the space environment, replacement of red cells in the crew of the 28-day mission was delayed for about two weeks. This "refractory" phenomenon was different from the more rapid recovery exhibited by the crews of the two longer missions as evidenced by a reticulocytosis and measured rise in red cell mass (Table 4 and Figure 24). It was shown previously that bed rest is characterized by a similar delay in red cell mass recovery as that seen after the 28-day space flight (Figure 6). However, a reticulocytosis was observed much earlier after bed rest than after space flights of similar duration (Dunn et al., 1977), even though red cell mass failed to show demonstrable repletion in both cases.

In the case of bed rest, a simulation analysis suggested the delay in red cell mass recovery may have resulted from a combination of factors which included a normal bone marrow transit time delay of several days, increased destruction of cells, decreased oxy-hemoglobin affinity, and a delay in plasma volume repletion (Kimzey et al., 1979; also see "Bed Rest Simulations.") The

last of these factors could not be adequately examined because of the sparsity of bed-rest data relating to plasma volume recovery. In Skylab, however, a much more complete description of plasma hemoglobin concentration changes (from which plasma volume could be inferred) was available during the postflight period. These data were presented in Figure 3 to 5 and have been redrawn in Figure 43 for convenience in comparing mission differences.

Accordingly, it appears that the hemodilution arising from plasma refilling was much greater following recovery day of the two longer missions, compared to the 28-day mission. This is confirmed, in part, by the less frequent plasma volume measurements which indicates that maximum repletion of plasma took place almost 6 weeks following recovery for the 28-day crew compared to two weeks for maximum recovery for the 59-day and 84-day crews (Figure 3, 4, and 5). No explanation for this difference in plasma refilling behavior has yet been advanced, but a tentative hypothesis is provided in the paragraph below.

If plasma volume losses were similar on all flights, it would be expected that the crewmembers with the largest decrements in red cell mass (i.e., the 28-day crew) would exhibit the greatest amount of postflight plasma volume refilling in order to return blood volume to the preflight level. However, inflight plasma volume changes were apparently not the same, but rather the least losses occurred on the 28-day flight and the greatest losses occurred on the 84-day flight. Also, the amount of postflight plasma volume refilling (by the end of two weeks postflight) was directly related to the amount of plasma lost inflight. The overshoot of plasma volume above control levels, often seen after bed rest and space flight, may be ascribed as a compensation for the previous losses in red cell mass. From the point of view that plasma volume is regulated both inflight and postflight to maintain blood volume appropriate to the respective gravity environments, the smaller degree of refilling on the 28-day mission seems more understandable.*

* The observation that the greatest losses in red cell mass were accompanied by the least losses in plasma volume resulted in a blood volume loss that was essentially constant regardless of mission duration (Fig. 2). This suggests a regulatory process whereby blood volume is maintained at reduced levels, appropriate for zero-g, by control of plasma volume. Accordingly, it can be presumed that downward plasma volume adjustments are required for the acute upper circulatory overload upon entering zero-g, and longer term upward adjustments take place in compensation for the more gradual red cell mass changes which would otherwise reduce blood volume further. Long-term plasma volume regulations in response to a primary disturbance in circulating red cell mass has also been noted in erythremic states such as polycythemia vera (Nusynowitze et al., 1974) and altitude hypoxia (Sanchez et al., 1970).

PLASMA [HB], PERCENT
CHANGE FROM PREFLIGHT

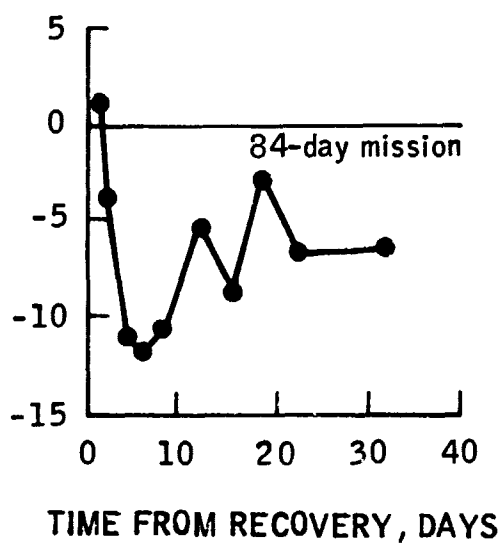
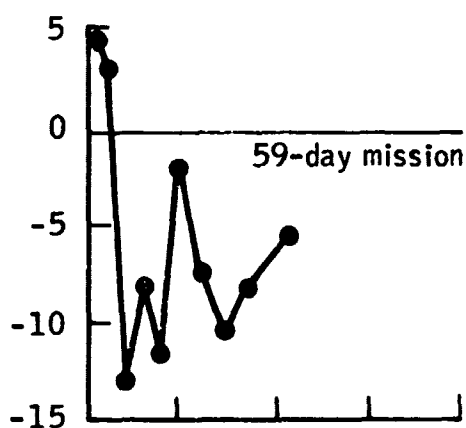
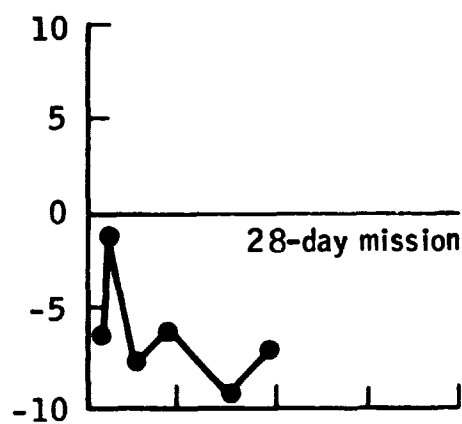


Figure 43: Postflight blood hemoglobin concentrations of each Skylab crew expressed as percent of preflight control.

The effect of plasma volume refilling following space flight was studied by computer simulation. An idealized space-flight simulation was performed by reducing the plasma volume 500 ml below control and permitting the model to achieve a new steady-state, at which time 250 ml of red cell mass had disappeared (see Figure 28). Thereafter, three idealized postflight cases were examined: plasma volume refilling of 250 ml (undershoot); plasma volume refilling of 500 ml (return to control); and plasma volume refilling of 750 ml (overshoot). The responses to these volume adjustments are shown in Figure 44. In each case, the model is responding to the simulated postflight hemodilution which has different levels of severity, depending upon the degree of plasma refilling. After 30 simulated days, the red cell mass had only returned to normal for the case where a plasma refilling overshoot was postulated (case C). For case A, where plasma refilling was incomplete, but hematocrit was depressed 6 percent from the last inflight day (similar to the 28-day mission), recovery of red cell mass was essentially negligible after two weeks, and after 30 days, only 33 percent of the lost red cells had reappeared in the circulation. Thus, when combined with the normal 4-day transit delay for bone marrow production (included in the model), the diminished recovery of plasma volume on the 28-day mission compared to that observed on the other flights seems sufficient to account for the differences in postflight recovery kinetics of red cell mass among crewmen. In addition, a small degree of hemolysis cannot be ruled out following the shortest mission (see Table 3). The available data do not permit any speculation regarding shifts in oxy-hemoglobin affinity, as was postulated in the bed-rest study (see "Bed-Rest Simulations").

Comparison Between Bed Rest and Space Flight

Comparison of the hematological responses between bed rest and space flight (i.e., Skylab) reveals many similarities, including: a) a gradual decrease in red cell mass, b) a more rapid decrease in plasma volume, c) hemoconcentration throughout the stress period, d) destruction rates of red cells which are either normal or not high enough to account for the total red cell mass loss, e) a depressed reticulocyte count immediately following the hypogravic stress which subsequently increases to supra-normal levels upon return to a one-g ambulatory environment, and f) a return-to-normal of red

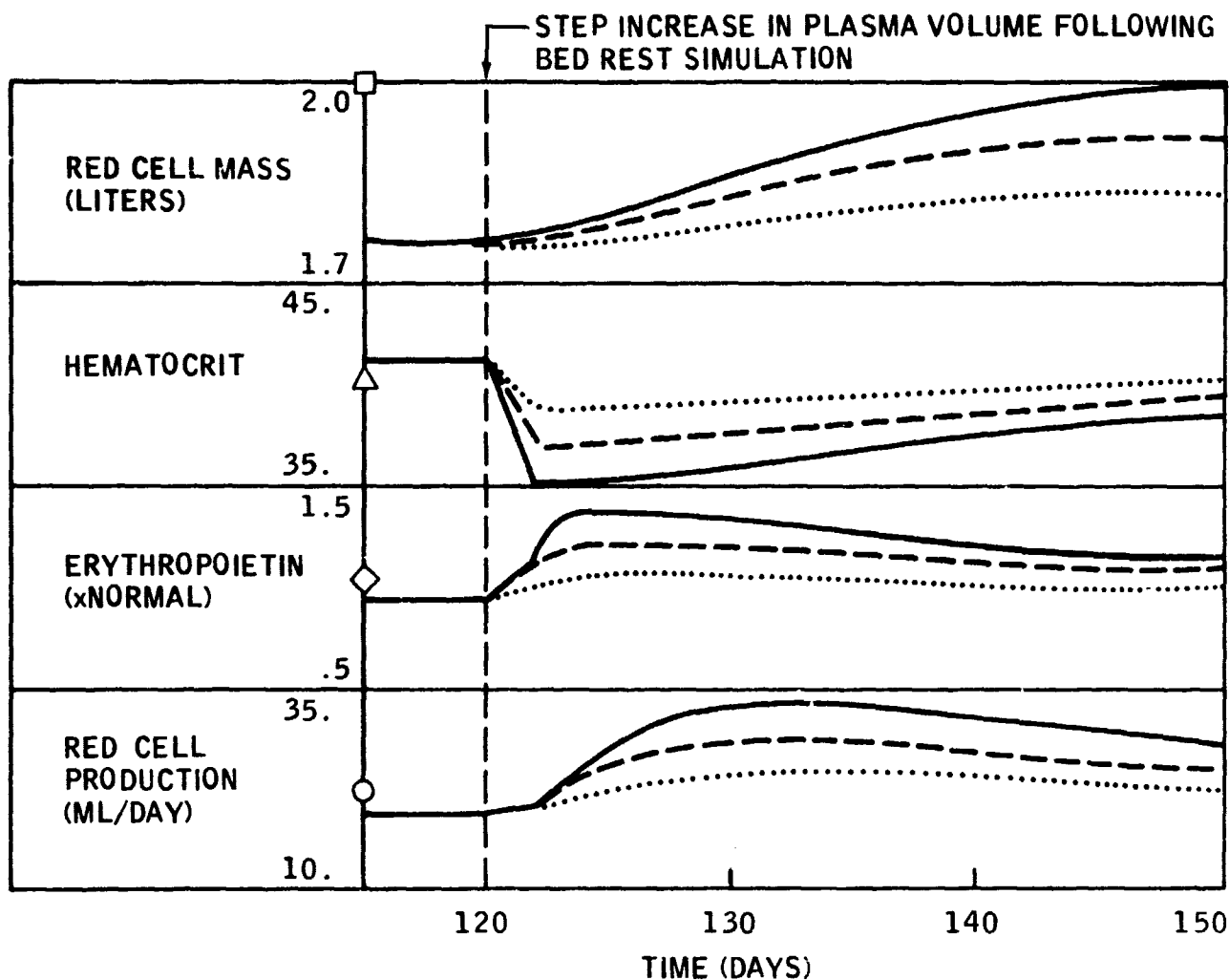


Figure 44: Simulation analysis of post bed-rest recovery of red cell mass showing the influence of different degrees of hemodilution. The responses shown in this figure were preceded by a 120-day bed-rest simulation (Fig. 28), which was accomplished solely by reducing plasma volume by 500 ml. The pre-bed rest control values are shown by the solid circles on the left and the end of bed-rest is indicated by vertical dashed line at 120 days. At 120 days, recovery was initiated by refilling plasma volume to various levels of repletion: Under-repletion, 250 ml added (.....), Complete repletion, 500 ml added (-----), Over-repletion, 750 ml added (———). The resulting hemodilution (indicated by the hematocrit response) then served as the erythropoietic stimulus for red cell mass recovery.

cell mass requiring 3-7 weeks. In addition, and as just discussed, a delay in red cell mass recovery has been found in those bed-rest and space-flight studies which last less than a month.

Blood volume losses for the first 30 days of space flight and bed rest are compared in Figure 45. The bed-rest results are based on two empirical formulations, which were derived from a large number of studies of different lengths: a) a predictive equation describing red cell mass loss was given as (Johnson and Driscoll, 1977):

$$\text{RCM Loss (percent)} = 0.24 \times \text{Days of Bed Rest} + 0.90$$

This formulation is based on bed-rest studies whose length varied from 2 to 35 days and should not be extrapolated outside this range; b) plasma volume losses were fitted by the following regression equation (Greenleaf, Bernauer, Young et al., 1977) which appears accurate for about 30 days of bed rest:

$$\text{PV Loss (percent)} = \text{Days of BR} / (0.33 + 0.039 \times \text{Days of BR})$$

The results from Gemini, Apollo, and the 28-day Skylab missions are taken from direct measurements on crewmen obtained during the first day of recovery. The other curves, representing the 59-day and 84-day Skylab missions, are taken from the computer simulations of Figure 42. These should be considered unconfirmed estimates, since no direct measurements of red cell mass and plasma volume are available in this time period. (The 28-day Skylab mission could not be accurately simulated since no inflight Hb data were obtained.)

Based on a strict interpretation of hard data (i.e., excluding estimates from computer simulation) it appears that losses of red cell mass are greater and plasma volume losses are smaller during space flight (solid line) as compared to bed rest (dashed line) (Johnson, 1979). Moreover, the differences between bed rest and space flight appear to become greater as a function of time. However, the data becomes more difficult to describe and generalize when one adds the observations from the two longer Skylab missions. First, there are no bed-rest studies of sufficient length with which to compare the 59-day and 84-day missions. Secondly, the space-flight data is most easily and superficially reconciled by inflight regeneration of red cell mass, but there are problems with this interpretation as discussed earlier in detail.

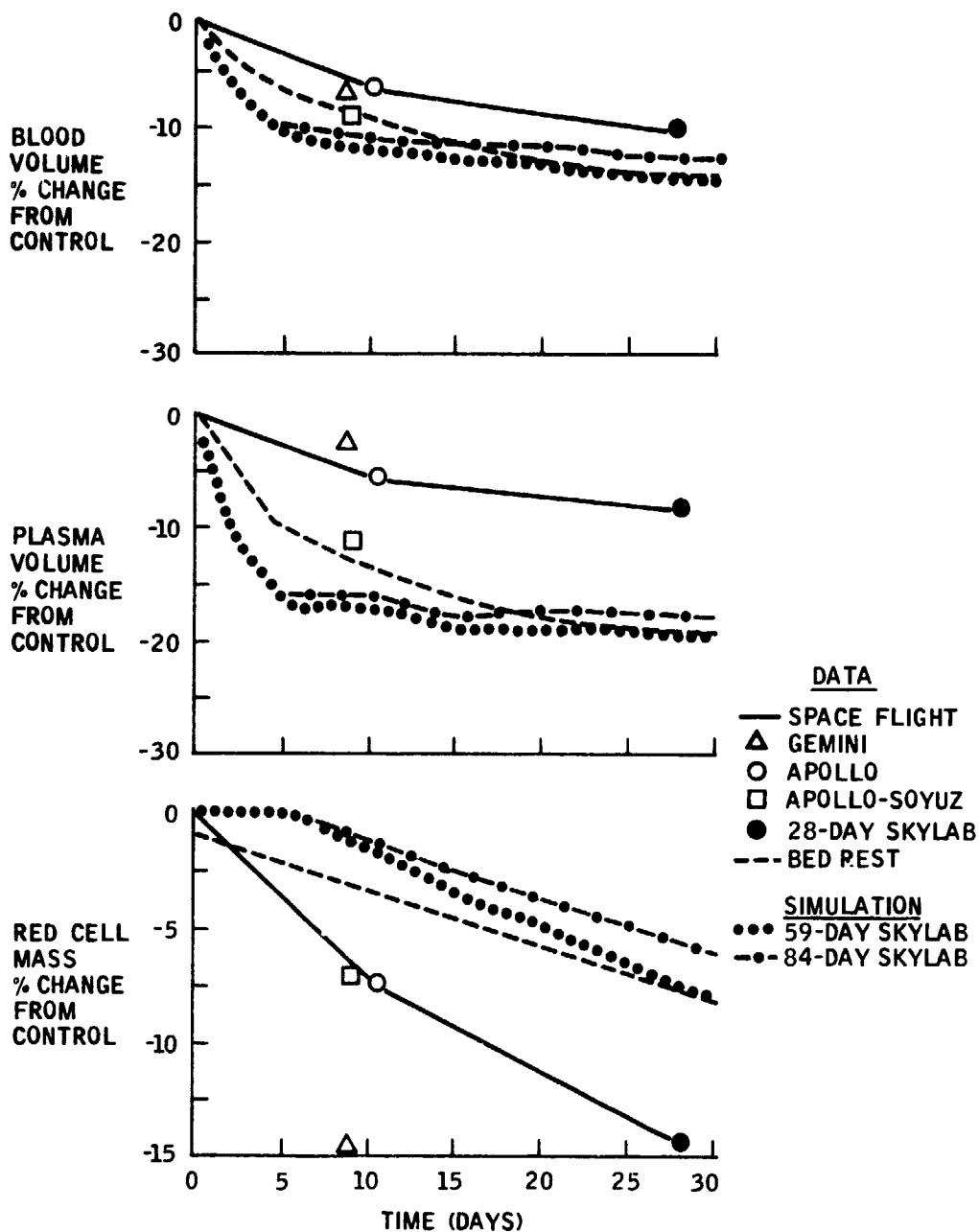


Figure 45: Changes in blood volume, plasma volume, and red cell mass during the first month of space flight and bed rest. Space-flight results are of two types: a) direct end-of-mission data (Kimzey, 1979) from combined-Apollo and the 28-day Skylab mission (solid line), and b) predictions from the simulation model for the first 30 days of the 59-day and 84-day Skylab missions (small open and filled circles) for which there is no direct data. Combined Gemini and Apollo-Soyuz results are included for reference only. Bed-rest data (dashed line) are derived from regression equations (see text) for plasma volume and red cell mass based on multiple studies.

Thirdly, if one tentatively accepts the computer simulation responses, there appear to be differences in rates of red cell loss among the three Skylab missions that are difficult to explain except on the basis of dietary and exercise regimens, the effects of which are presently not well understood. The simulation responses shown in Figure 45 suggest that the two longer Skylab missions and bed rest are more nearly similar to each other, especially for prolonged flights, and that the space missions lasting less than one month are the exception. This last interpretation can be defended on the grounds that the shorter flights were distinguished by some evidence of intravascular hemolysis.

The simulated responses also suggest a greater and more rapid fall in plasma volume during space flight than occurs either in bed rest (dashed line) or is suggested to occur from the first-day-of-recovery space-flight data (solid line). The simulations may, in fact, be a reasonable reflection of the truth, for several reasons. The simulations were inferred from inflight hemoglobin values which exhibited rapid increases (and of greater magnitude than in bed rest) and indicate corresponding decreases in plasma volume (compare Figure 6 and 23). On the other hand, direct measurements of plasma volume on the day of recovery may not be representative of the true inflight loss because of the body's capacity to rapidly replenish plasma deficits. Also, the possibility exists that space flight plasma volume losses were underestimated because of a diurnal variation (Johnson, 1979).

One of the more intriguing patterns that emerges from Figure 45 is that associated with the blood volume data. Compared to the results of red cell mass and plasma volume, the blood volume losses appear to be more uniform (i.e., less scatter) for all bed-rest and space flights examined. This observation adds support to the concept, previously stated, that total blood volume is closely regulated and tends to stabilize at a level approximately 90 percent of control.

If hemoconcentration resulting from plasma volume depletion was the sole cause of red cell mass change, it would be expected that those situations in which plasma volume dropped more rapidly (i.e., 59-day and 84-day Skylab missions) would be associated with the most rapid drop in red cell mass. This hypothesis is supported by the idealized simulation shown in Figure 46 in which plasma volume is assumed to decrease either rapidly (as in space flight) to a constant level, or falls more gradually (as in bed rest) to the same

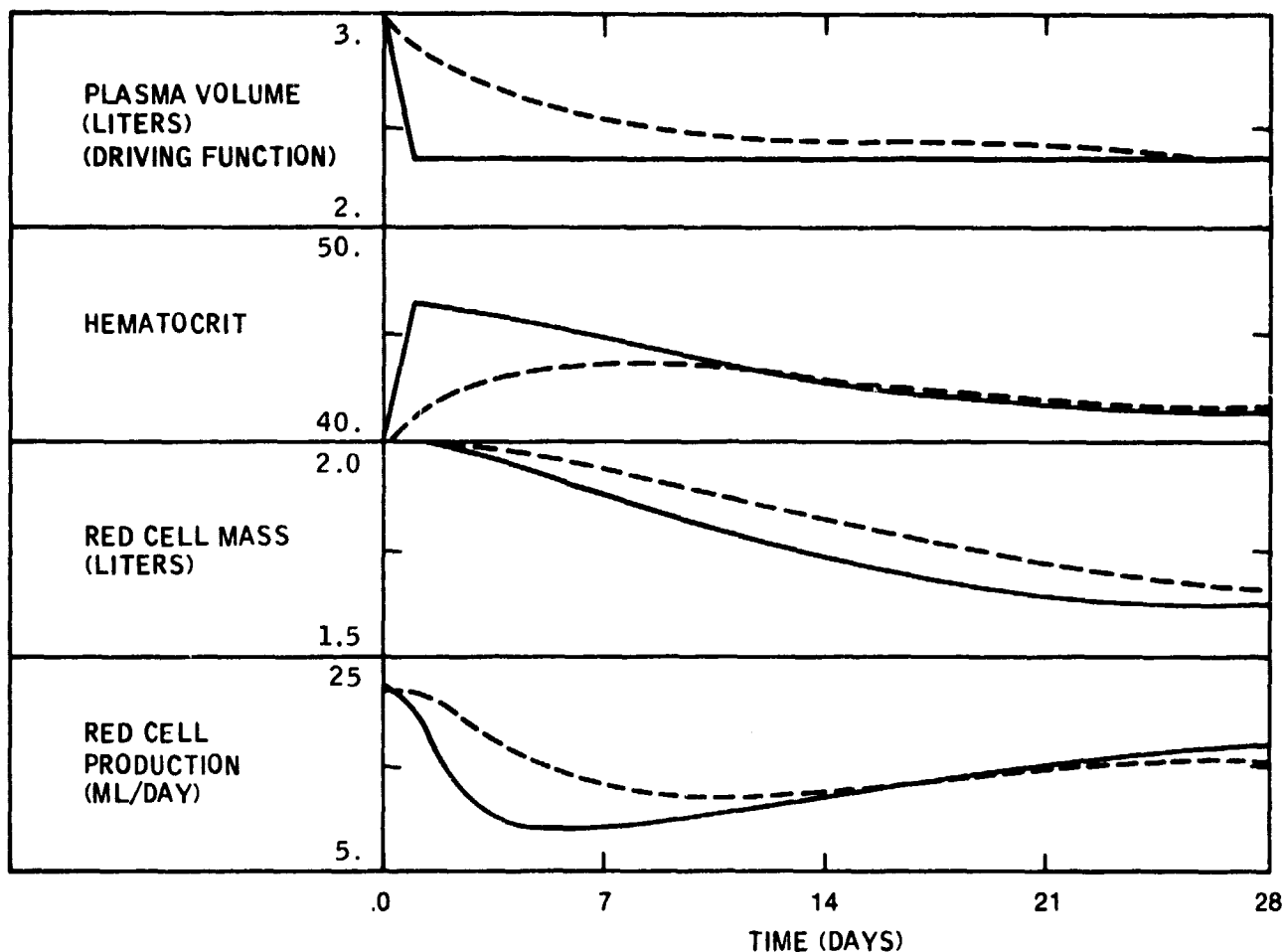


Figure 46: Simulation of an acute drop in plasma volume (representing space flight) in comparison to an exponential fall in plasma volume (representing bed rest). Erythropoiesis is controlled by the hematocrit-time profile. On the basis of a hemoconcentration influence alone, the model predicts a greater erythro-suppression for space flight than for bed rest during the first 4 weeks. Because plasma volume decreases to the same level in both cases, convergence of all corresponding variables also occurs near the end of the simulation.

level. However, the contrary situation seems to be present in Figure 45; i.e., those studies in which plasma volume decreased most rapidly were accompanied by the least changes in red cell mass. This does not negate hemoconcentration as a causative agent in red cell loss, but suggests that other factors were present to varying degrees in the cases examined in Figure 45. In this regard, and in the light of the previous discussions on the role of energy balance, the differences between diet and overall metabolic rate between space flight and bed rest merits further attention. Bed-rest subjects are often given a decreased dietary intake thought to be commensurate with the decreased metabolic rate. Twenty-four hour metabolic rates during space flight have not been measured, but they undoubtedly have varied widely as a result of nearly total confinement in the earlier missions and the free-ranging movement in the lunar environment and the Skylab workshop. This has been accompanied by a nearly universal occurrence of inadequate dietary intake, with the single exception of the 84-day mission.

The general conclusion appears inescapable that bed rest and space flight have more similarities than differences with regard to the erythropoietic response. However, a more complete description of the disturbances in oxygen transport, bone marrow activity, and red cell lifespan are required in both situations. Differences between bed rest and space flight may ultimately be explained by the energy balance factors of diet and metabolic rate, and by the dynamics of plasma volume shifts.

SUMMARY

The regulation of erythropoiesis during weightless space flight was studied using a theoretical model. The model incorporates the best current understanding of the dynamics of red cell production and the associated feedback regulation based on tissue oxygenation. Using the techniques of computer simulations, it was possible to apply the model to study candidate hypotheses which might explain the loss of circulating red cell mass during space flight.

Both ground-based and space-flight experiments have suggested that, in the normoxic environment of Skylab, this loss was likely a result of suppressed erythropoietic activity rather than elevated destruction of red cells. According to current concepts embodied in the theoretical model, a reduced erythropoietic state can be caused indirectly by hyperoxia of a renal oxygen

sensor via the humoral regulator, erythropoietin, acting on the bone marrow, or by direct alteration of stem cell kinetics at the marrow controller. (The presence of erythropoietin inhibitors was not considered in this analysis). Tissue hyperoxia may be caused by factors which increase oxygen supply (i.e., hemoconcentration, enhanced blood flow, decreased oxy-hemoglobin affinity, and increased arterial oxygen loading), or by factors which decrease oxygen demand. Proliferation of erythrocytes at the bone marrow level is affected, not only by erythropoietin, but also by dietary factors. One can reasonably postulate that all of these various influences were present to various degrees during space flight and bed rest, and they contributed to the eventual reduction in red cell mass.

Evaluation of these hypotheses was accomplished by several means including: sensitivity analyses which revealed the relative influence of each major hematological parameter on circulating red cell mass; dynamic simulation of human and animal studies, whereby the effects of single, multiple, or time-varying stresses were assessed for their potential to generate computer responses similar to those observed experimentally; and, collaboration with investigators performing animal studies to test, in the biological system, those hypotheses suggested by the computer model. Results from a number of experimental studies, each characterized by a reduced red cell mass or suppressed erythropoietic activity, were examined. These included not only space-flight investigations, but also those of bed rest, red cell infusions, dehydration and descent-from-altitude. The simulation model was valuable in revealing the pathways which were common to all of these situations and provided a quantitative basis for testing whether the same mechanisms were operative in space flight.

This hypothesis testing approach led to the following conclusions and hypotheses which are summarized in Figure 47:

a) The shifts in plasma volume accompanying hypogravic maneuvers results in an observed mild hemoconcentration which can eventually lead to significant decrements in circulating red cell mass. The model predicts that moderate increases in hematocrit, if unopposed by other factors involving oxygen delivery to tissues, can proportionately increase oxygen tension at a renal sensing site and exert a sensitive suppressant effect on erythropoietin and red cell production. The erythropoietic regulatory system may be viewed, when

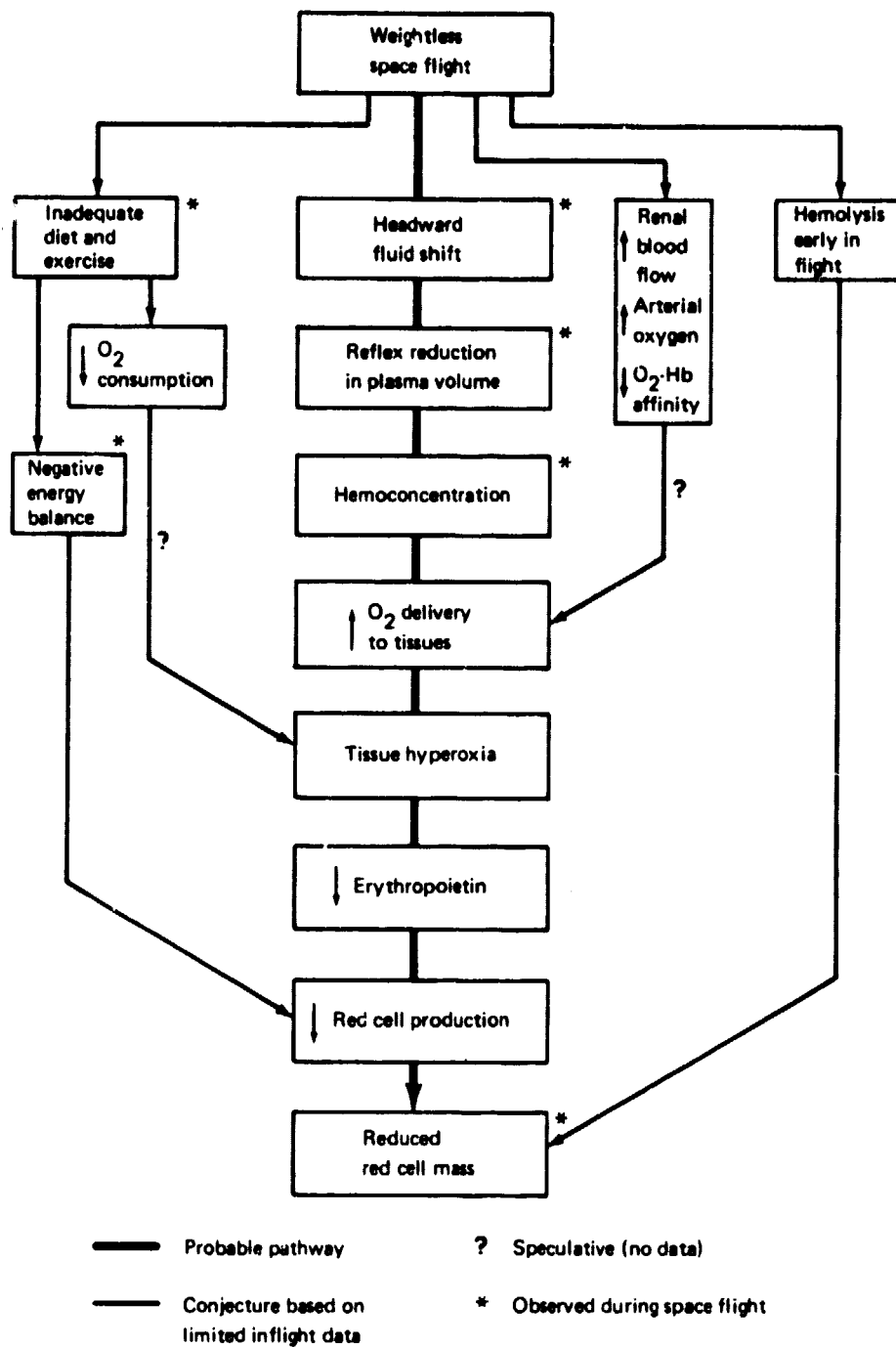


Figure 47: Pathways which may be involved in the loss of red cell mass during space flight.

operating in this fashion, as a hemoglobinometer; i.e., red cell production decreases so as to eventually relieve the hyperoxic condition. The final predicted result is a nearly complete restoration of hematocrit accompanied by a diminished red cell mass. The simulation of bed rest, using the observed non-linear plasma volume changes as model driving functions, predicted a qualitatively accurate linear rate of fall in red cell mass. The model suggests that red cell mass will stabilize as hematocrits normalize; this has not yet been confirmed experimentally. This proposed process can, therefore, be explained in terms of normal feedback regulation of the erythropoietic system in the face of sustained decreases in plasma volume.

b) Other factors which may have enhanced oxygen delivery and produced the same effects as hemoconcentration cannot be ruled out (i.e., shifts in blood flow, P_{50} , and arterial pO_2), but direct data are only available to support the hemoconcentration effect. In any event, the model system predicts that even small changes in a variety of parameters (perhaps smaller than can be measured experimentally), if sustained for long enough periods of time, could lead to a progressive and significant degeneration of red cell mass.

c) The ability of hemoconcentrated blood to suppress erythropoietic activity was qualitatively similar in all situations studied, whether it involved red cell infusion, descent from altitude, water deprived dehydration, bed rest, or space flight. However, as revealed by computer simulation, the quantitative effect was not always the same. That is, equal degrees of hemoconcentration did not result in the same degree of erythro-suppression for bed rest and space flight, nor for the 59-day and 84-day Skylab missions. This suggested that other factors may have been present which either augmented or opposed the hemoconcentration effect.

d) Two additional factors were examined: diet and exercise. The hypotheses that dietary restriction can reduce, and exercise can enhance, erythropoiesis (as reported in animal studies) appeared to account for the discrepancies noted in (c). An inadequate diet, in particular, has been shown to lead to suppressed erythropoiesis by mechanisms which may bypass the hormone regulator and act directly on the bone marrow controller. This could

explain the occasional findings that erythropoietic activity decreases in the presence of normal levels of erythropoietin. However, both dietary and exercise effects have not been well defined in the human subject and the lack of appropriate controls during space-flight studies does not permit a definitive conclusion.

e) The simulation analysis failed to rule out some small degree of acute red cell destruction as contributing to either the reduced red cell mass during hypogravity or the delayed recovery of red cells following some space flights and bed rest. Unusual removal of cells from the circulation could possibly arise from the mechanical stresses of launch, splenic sequestration, exercise hemolysis during flight, or postflight hemolysis of cells made more fragile by weightlessness or bed rest. However, a major role of inflight cell destruction is unlikely, because this would contradict the observations that hemoglobin concentrations are significantly elevated and that overt signs of hemolysis were seldom found.

f) The model erroneously predicts an identical hematological response to both relative (RP) and absolute (AP) polycythemia (produced by plasma volume decreases or red cell mass increases, respectively). While both cases result in hemoconcentration and reduced erythropoietic activity, the influences of blood volume (i.e., RP is hypovolemic and AP is hypervolemic) on blood flow which is not accounted for in the model may partially explain the different experimental responses to these two stresses.

g) Two theories were examined to explain the Skylab findings which demonstrated decreasing red cell mass losses with increased exposure to weightlessness. According to the so-called "regeneration theory," this behavior represents an adaptive influence to an initial exposure to weightlessness. Treating the Skylab crew data from three separate missions as a composite suggested that an early loss of red cells is followed by regeneration which begins two months after launch. Acceptance of this hypothesis as a generalized theory of erythropoietic regulation in weightlessness is confounded by the fact that decreasing losses of red cell mass were associated, not only with longer duration flights, but also with

increasing levels of diet and exercise. These latter factors had demonstrable effects on maintenance of body tissue and cardiovascular condition, and their further involvement in the oxygen transport-erythropoietic system was postulated herein. As a result, an alternative and more plausible ("continuous loss") theory was proposed which suggested that there were two components to the suppression of red cell production: one related to energy balance and one related to water balance. Differences among the crewmen's red cell mass losses were thereby considered to be a result of different levels of dietary intake and exercise, superimposed on a common loss due to hemoconcentration. According to this concept, the kinetics of red cell mass disappearance would not include regenerative behavior, but would be more similar to the continuous, linear losses observed in bed rest.

h) Computer simulation predicted that in most cases the observed repletion of red cells during the recovery period from hypogravity can be attributed to hemodilution-induced hypoxia (i.e., an effective anemia resulting from inflight red cell mass depletion and followed by postflight plasma refilling). The delayed recovery of red cell mass during the 28-day Skylab mission and the 28-day bed-rest simulation of that mission was attributed in part to a normal 3-4 day bone marrow transmit time and a time-lag in plasma volume recovery. The presence of an acute reticulocytosis following bed rest and its absence after a Skylab mission of the same length suggests somewhat different mechanisms were operative in each case. The involvement of shifts in oxy-hemoglobin affinity, mild hemolysis, or ineffective erythropoiesis were suggested as areas of future study.

i) The hematological responses to bed rest and space flight were found to be more similar than different. Common characteristics of both stresses include the loss of red cell mass, loss of plasma volume, hemoconcentration, and, in some cases, the kinetics of recovery. In addition, the restricted dietary intake and diminished metabolic demands of bed rest are qualitatively similar to that exhibited on the shorter space flights. However, the plasma volume losses may not occur in bed rest as rapidly as observed in space flight and the dynamics of hemoconcentration are somewhat different. Also, it is possible that the history of exercise during space flight compared to the

history of inactivity during bed rest may be partly responsible for different kinetics of the recovery response. It is probable that at least two elements, a negative energy balance and negative water balance, may be important causative factors in both bed rest and space flight.

j) The red cell mass losses on the earlier space flights were attributed to the toxic effects of 100 percent oxygen atmosphere and a resulting intravascular hemolysis. The results of the present study suggest that many of the factors considered to explain the Skylab and bed-rest observations, particularly, hemoconcentration, negative energy balance, and decreased physical activity undoubtedly also played a contributory role in the Gemini and Apollo flights. This helps to explain the large red cell mass losses, observed during these relatively short flights.

The discussion in this section extends the analysis of the hematological responses originally presented in the Skylab postflight reports (Kimzey, 1977; Johnson and Driscoll, 1977). The lack of additional space-flight experimentation in the intervening period precluded testing of newly developed hypotheses in a true weightless environment. Some of the experimental discrepancies revealed by the quantitative nature of model simulation analysis suggest approaches for future experimentation. For example, it is crucial to obtain direct inflight measurements of erythropoietin and bone marrow activity. If reduced erythropoietin levels cannot be demonstrated during space flight, then the hemoconcentration theory may have to be abandoned. (The first inflight measurements of erythropoietin were recently performed (Leach and Johnson, 1984) and showed a statistically non-significant decrease in this hormone). Also, the importance of diet and exercise on erythropoiesis needs to be studied more carefully in humans with and without accompanying hypogravity. The question has been raised whether moderate and relatively short periods of dietary or exercise restriction by themselves could alter red cell production in humans. At the very least, diet and exercise should be controlled more carefully on future missions in which the hematological system is under investigation. The present analysis has also questioned the occurrence of inflight regeneration of red cell mass. Testing the regeneration hypothesis will require inflight measurements of red cell mass on flights lasting several months. While no U.S. manned flights of this duration are planned for the

near future, data from the long-term Soviet mission does not support the regeneration concept (Johnson, 1983). In addition to inflight studies, the question of postflight kinetics was not entirely resolved. For example, it is not simple to explain the presence of a reticulocytosis during the immediate post-bed-rest period in contrast to a delayed reticulocytosis in a space flight of comparable duration. Also, the profound influence that oxygen-hemoglobin affinity might exert on inflight and postflight tissue oxygenation as predicted by the model is worthy of additional attention.

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